RADIOACTIVE INDICATORS

Their Application in Biochemistry,
Animal Physiology, and Pathology

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GEORGE HEVESY

1 9 4 8

To
AUGUST KROGH

PREFACE

This is a survey of much work carried out with radioactive indicators in the fields of animal physiology, pathology, and biochemistry. A separate volume is contemplated on the application of radioactive tracers in plant physiology. Although no fundamental difference is to be found between the applications of radioactive and of stable isotopes as tracers, the scope of this volume is limited to the radioactive indicators. In view of the increasing use of tracer methods it is very possible that not many surveys will appear in the future, but the results obtained by means of radioactive indicators will be incorporated into textbooks dealing with the over-all aspects of the specific topics concerned. At present, however, it seems justified to offer a collective presentation of the results.

This volume was written in the Institute for Theoretical Physics of the University of Copenhagen and the Institute for Research in Organic Chemistry of the University of Stockholm; it was completed in the Donner Laboratory of the University of California. The author wishes to express his thanks to the directors and staffs of these institutions for their continual encouragement. He feels, furthermore, much indebted to Dr. W. Armstrong, Dr. Dorothy Axelrod, Dr. H. A. Barker, Dr. I. L. Chaikoff, Dr. D. H. Coop, Dr. R. Craig, Dr. F. O. Fenn, Dr. D. M. Greenberg, Dr. G. J. Hamilton, Dr. H. Kalckar, Dr. A. Krogh, Dr. L. Marinelli, Dr. Lise Meitner, Dr. F. Paneth, Dr. E. Segrè, Dr. H. Tarver, Dr. A. Tobias, Dr. R. E. Yankevich, Dr. L. Zechmeister, and Dr. D. B. Zilversmit for valuable suggestions and the permission to communicate hitherto unpublished results, diagrams, and radiograms. Dr. L. J. Mullins, Dr. J. Ottesen, and Dr. K. Zerahn kindly read the proofs. Miss S. Hellmann rendered much technical assistance. Miss Clara J. Douglas prepared the subject index.

Thanks are also due to Interscience Publishers for their understanding cooperation. Miss Miriam T. Malakoff and Mrs. Arthur Kornberg, the editors of this volume, made great efforts to improve its contents.

The Segrè chart is included through the kind permission of Dr. Segrè and of Addison-Wesley, Inc., Cambridge, Mass.

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June, 1948

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Production of Radioactive Indicators

I. General Remarks

In many of the investigations carried out with radioactive tracers it is important to use an indicator of negligible weight, *i.e.*, of very high specific activity. In investigating the turnover of iodine, for example, administration of as little as 1γ iodine to the rat creates nonphysiological conditions, and it is therefore imperative to use an almost infinitesimal amount of labeled iodine; carrier-free radioiodine of great activity is supplied by the Atomic Energy Commission (see Table 3). In investigations with labeled phosphorus, the presence of some ^{31}P in the preparation is often without importance in view of the appreciable inorganic phosphorus content of plasma and the rapid interchange of plasma phosphate with the inorganic and labile phosphate of liver and other viscera. No difference in ^{32}P recovery in such tissues as brain and blood was observed when such widely differing amounts of labeled phosphorus as 6.0 and 0.3 mg. were injected into 200-g. rats.[1] However, in experiments with ^{32}P, especially when the phosphate is injected into the circulation, a sudden increase in the phosphate concentration of the plasma may have disturbing effects.

If a cyclotron is available a neutron stream of great intensity can be obtained by bombarding beryllium with the accelerated heavy particles in the cyclotron. But even heavier elements, when bombarded by such particles of high energy, supply a copious source of neutrons. A still more powerful source of neutrons is the pile. By making use of these neutrons, carrier-free radioactive isotopes of many elements can be obtained. It is very convenient to use neutron sources composed of a mixture of radium salt and beryllium, although the intensity of the neutron beam emitted by a source containing as much as 1 g. radium is much less than the intensity obtained by an effective high-voltage aggregate and very much less than that obtained by the cyclotron, not to mention the pile. The activity of ^{32}P obtainable by the action of

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[1] B. A. Fries and I. L. Chaikoff, *J. Biol. Chem.*, **141**, 469 (1941).

neutrons emitted from a neutron source containing 1 g. radium is about five microcuries. Most neutrons emitted by the radium source are produced by the reaction:

$$\_4^9Be + \_2^4He \rightarrow \_6^{12}C + \_0^1n$$
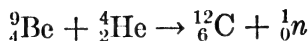
Such sources are most conveniently prepared by mixing powdered beryllium with radium chloride or radium sulfate (1 g. radium salt with 5 g. beryllium). These mixtures do not require renewal, in contrast to mixtures of beryllium powder and radon, which lose half their strength in the course of 3.8 days. Neutrons produced in this manner have a more or less continuous distribution in energy extending up to about 13 m.e.v. for radon alpha particles and somewhat less for radium alpha particles. The radioelements produced by neutron bombardment will be diluted invariably by an overwhelming mass of inactive material, and for their use as indicators it is necessary to extract and concentrate them.

II. Preparation of Radiophosphorus

If a radioactive isotope is produced by bombardment of a different element with neutrons or other energetic particles, the separation of the radioelement can usually be carried out by ordinary chemical methods. ^{32}P, for example, can be produced by bombarding carbon disulfide[2-4] with fast neutrons according to the equation:

$$\_{16}^{32}S + \_0^1n \rightarrow \_{15}^{32}P + \_1^1H$$
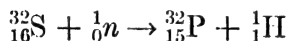
The neutron source is placed in the center of a flask containing about ten liters of carbon disulfide. The liquid is later treated with water or dilute acids in which ^{32}P is soluble, while carbon disulfide is not. By prolonged shaking an almost quantitative separation can be obtained. The dilute acid or the water used to extract the radiophosphorus should contain no other phosphorus than the ^{32}P and possibly some ^{31}P present in the carbon disulfide irradiated. By repeated irradiation of the carbon disulfide, followed by extraction of the radiophosphorus produced, the soluble impurities present in the carbon disulfide are, however, successively eliminated.

[2] O. Chievitz and G. Hevesy, *Nature*, **136**, 754 (1935). *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **XIII**, 9 (1937). A. J. Alichanow, A. J. Alichanjan, and B. S. Dzelepow, *Phys. Z. Sowj.*, **10**, 78 (1936).

[3] J. Govaerts, *Bull. soc. roy. sci. Liége*, **8**, 121 (1939); *Nature*, **141**, 871 (1938).

[4] O. Erbacher and K. Philipp, *Ber.*, **69**, 893 (1936).

Radiophosphorus produced by the action of fast neutrons on carbon disulfide can also be collected in an electric field.[5] Copper electrodes are placed in a liter of carbon disulfide under neutron bombardment; by the action of an electric field (for example, 100 v. per cm.), the ^{32}P produced migrates to the electrodes and is deposited on these. The anode is found to contain about one and one-half times more ^{32}P than the cathode, the total yield of deposition being about 95%. The copper electrodes are later dissolved and the copper is precipitated as copper sulfide, while ^{32}P remains in solution.

Decay of the radioelements follows the exponential law. The number of atoms disintegrating per unit time is proportional to the total number of the atoms of the radioelements and, thus, the number present after the lapse of t time units, N_t, is $N_0 e^{-\lambda t}$, where N_0 is the number of atoms initially present and λ is the decay constant. Instead of this constant, the half-life, T, is often stated. $T = 0.69/\lambda$ and denotes the time it takes for half the atoms originally present to disintegrate. Table 16 (page 81) shows the rate of disintegration of radiophosphorus, which has a half-life of 14.3 days, while in Tables 14 and 15 the rates of disintegration of ^{24}Na and ^{42}K are recorded.

The growth of radioactivity when an element is under constant bombardment follows the familiar growth law, i.e., the fraction of the saturation number of atoms formed in a half-lifetime t is equal to $1 - e^{-0.69 t/T}$. Neutron bombardment of sulfur (or a sulfur compound) for 14.3 days yields half the maximum amount of radiophosphorus. After the lapse of 2×14.3 days, three-fourths the maximum amount is obtained. We often content ourselves with about one-half the maximum amount obtainable instead of extending the bombardment over many weeks, which is obviously not economical.

When the irradiation of liquid sulfur compounds is to be avoided,* elementary sulfur is exposed to the action of neutrons, and the ^{32}P is isolated in a way devised by Cohn.[6] 1.5 to 2.5 kg. sulfur is kept overnight at 120–130° C.; the molten sulfur is then poured into 4 liters of concentrated nitric acid kept at 90°C. After the solution is stirred for 1–2 minutes it is filtered through a glass wool plug. The filtrate obtained contains, beside the radiophosphoric acid, about 200–300 ml. concen-

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* As is the case in the pile.

[5] J. Govaerts, *J. chim. phys.* **36**, 130 (1939).

[6] W. E. Cohn, *Isotope Branch Circ. No. C-1, U. S. Atomic Energy Commission*, Jan. 7, 1947.

trated sulfuric acid formed in the course of the reaction, and nitric acid, which is removed by boiling. The further steps depend on whether or not the solution contains, as it occasionally does, aluminum or chromium. If these impurities are absent the following procedure is chosen:

(a) After adding 50 mg. ferric chloride, iron and radiophosphorus are precipitated by adding ammonia. After centrifuging, the supernatant fluid is discarded. To remove contaminating sulfur and sulfur compounds, the precipitate is dissolved in dilute hydrochloric acid; ammonia is added and the precipitate obtained dissolved again in hydrochloric acid. After three treatments of this type, the solution is found to be free of sulfur and sulfur compounds. Before separating the radiophosphorus from iron 40 mg. phosphoric acid are added as carrier; then, by shaking the 8 N hydrochloric acid solution containing iron and phosphorus with isopropyl ether, the iron is extracted. This procedure has to be repeated three times. After evaporating to dryness the phosphoric acid is taken up with water and neutralized with sodium hydroxide to pH 7.5.

(b) When aluminum or chromium is present in the solution the centrifuged precipitate containing iron and radiophosphorus is dissolved in 0.3 to 0.4 N nitric acid; 40 mg. phosphoric acid is added and the phosphorus is precipitated as ammonium phosphomolybdate. Two solutions are prepared. Solution 1 is an ammoniacal solution of ammonium molybdate made by dissolving 100 g. ammonium molybdate (or molybdic oxide) in 400 ml. water and 80 ml. 15 M ammonium hydroxide. Solution 2 is made by mixing 400 ml. 16 M nitric acid with 600 ml. water. The two solutions are kept separate and mixed only in such portions as are needed for immediate use, the necessary amount of solution 1 being poured into twice its volume of solution 2, and the resultant solution being then added to the solution containing the phosphorus. Thus 10 ml. of solution 1 corresponds to 2 g. ammonium molybdate and this amount should be used with 20 ml. of solution 2 for any amounts of phosphorus up to 20 mg. For larger amounts of phosphorus, correspondingly greater amounts of the two solutions must be used. It is to be noted that ammonium molybdate reagent should always be added in decided excess, namely, about 2 g. ammonium molybdate over the amount calculated (1 g. ammonium molybdate corresponds to about 0.150 g. phosphorus).

In the precipitation of ammonium phosphomolybdate, attention

must be given to the following essential details. The solution, before the addition of the molybdate reagent, should be acidified slightly with nitric acid, to a pH somewhere between 4.0 and 5.0. It should contain a relatively high concentration of ammonium nitrate, *i.e.*, 0.5 M (4 g. ammonium nitrate in 100 ml.). The temperature at the time of precipitation should be about 40°. The ammonium molybdate reagent should be added rapidly with constant stirring and in the excess previously stated. The solution should be set aside and the precipitate of ammonium phosphomolybdate allowed to digest overnight.

The ammonium phosphomolybdate precipitate is dissolved in 30 ml. warm 2.5 M ammonium hydroxide, the solution made 1 N with hydrochloric acid and ammonium sulfide added to remove the molybdenum. The filtrate is then heated with hydrochloric and nitric acids until all the ammonium salts are volatilized. The residue is then taken up with water and brought to pH 7.5 by adding sodium hydroxide.

When radiophosphorus is prepared by deuteron bombardment (for yields, see page 40), iron phosphide is often used as the internal target substance. When the target material can be bombarded inside the cyclotron vacuum chamber, much larger beams can be employed and these beams can be concentrated over a very small area. Phosphide is dissolved in concentrated aqua regia and the method described is applied to the isolation of phosphorus. As the target contains an appreciable amount of phosphorus (about 50 mg.) no carrier has to be added in the course of purification. A convenient method of preparation of ^{32}P applied at the Nobel Institute of Physics is the bombardment of phosphorus pentoxide with deuterons. Since the phosphoric acid obtained after dissolving the phosphorus pentoxide in water is mostly contaminated with traces of copper originating from the target, after addition of a few milligrams of inactive carrier copper hydrogen sulfide is passed through the solution to remove these traces. The supernatant is boiled to remove hydrogen sulfide. It is then titrated into sodium hydroxide to the phenol red point; one ml. of normal hydroxide is equivalent to 71 mg. sodium monohydrogen phosphate. In many investigations, phosphorus is administered as sodium monohydrogen phosphate of almost negligible weight dissolved in physiological sodium chloride solution. It is sometimes required that the phosphate solution, as Na_2HPO_4, be isotonic. This corresponds to 3.3 mg. P, or 14.8 mg. Na_2HPO_4, per ml. The percentage of ^{32}P content, even of phosphorus of high specific activity, is extremely low for the most part. 1 mg.

phosphorus of 1 millicurie activity contains only 3.4×10^{-6} mg. ^{32}P. This figure is derived from the ratio:

$$\frac{\text{half-life of } ^{32}\text{P (days)}}{\text{half-life of Ra (days)}} \times \frac{\text{atomic wt. of } ^{32}\text{P}}{\text{atomic wt. of Ra}} = \frac{14.3}{572,400} \times \frac{32}{226}$$

III. Production of Radioarsenic

Radioarsenic, $^{74}_{33}$As, with a 16-day half-life can be produced by the reaction:

$$^{73}_{32}\text{Ge} + {}^{2}_{1}\text{D} \rightarrow {}^{74}_{33}\text{As} + {}^{1}_{0}n$$
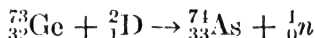
The arsenic so formed (for yield, see page 40) is in a mixture of germanium, copper, tin, lead, and minor impurities. To free it from these impurities and reduce the element to a form in which it can be used, the following procedure is used. The target is dissolved in aqua regia; some carrier arsenic is added, and the solution is evaporated to dryness. This removes the excess nitric acid and most of the germanium. Concentrated hydrochloric acid and a small amount (5 ml.) of concentrated hydrobromic acid are added and distilled into a chilled receiver. The hydrobromic acid reduces As^{5+} to As^{3+}, which passes over with the hydrochloric acid and bromine as $AsCl_3$. This distillation is repeated after the addition of more acid to the residue. To the combined distillates an excess of ammonium hypophosphite is added, and the solution is warmed to 90° C. for five minutes. Metallic arsenic precipitates, and is filtered out through a porcelain microfilter crucible. From this point the arsenic can be converted to any form suitable for its use. Yields of 65–75% are obtained by this method when 1 to 10 mg. carrier is used.[7,8]

When carrier-free radioarsenic is wanted, the germanium target of the cyclotron is dissolved in concentrated hydrochloric acid and the germanium tetrachloride is distilled off in a stream of chlorine; the arsenic is present in pentavalent state, which is not volatile. When all germanium is removed, the chlorine stream is shut off, hydrogen bromide is added to destroy the remaining chlorine and to reduce the pentavalent arsenic. The arsenic, now in the trivalent state, is distilled off and is collected in nitric acid. After evaporation of the nitric acid the invisible amounts of ^{74}As are dissolved in 0.9% sodium chloride solution and injected.[9] See also page 11.

[7] J. W. Irvine, Jr., *J. Applied Physics*, **12**, 347 (1941). *J. Phys. Chem.*, **46**, 910 (1942).

[8] J. N. Wilson and R. G. Dickinson, *J. Am. Chem. Soc.*, **59**, 1358 (1937).

[9] H. Lanz, personal communication.

IV. Production of Tracer Sulfur

The most important reactions leading to the formation of radio-sulfur (^{35}S) may be written as follows:

$$^{34}_{16}S + {}^{2}_{1}D \rightarrow {}^{35}_{16}S + {}^{1}_{1}H \qquad (a)$$

$$^{37}_{17}Cl + {}^{2}_{1}D \rightarrow {}^{35}_{16}S + {}^{4}_{2}He \qquad (b)$$

$$^{35}_{17}Cl + {}^{1}_{0}n \rightarrow {}^{35}_{16}S + {}^{1}_{1}H \qquad (c)$$

Method c proves to be the most efficient of the three. Both the cross section and the isotope abundance (75%) were favorable, as shown by Kamen,[10] for obtaining large quantities of ^{35}S. About 10–20 liters of carbon tetrachloride is bombarded with fast neutrons.[10] After the bombardment, some sulfur and 100–200 ml. of a solution of 1 N sodium hydroxide containing 1 ml. bromine are added. After refluxing the carbon tetrachloride with alkaline hypobromite for two hours, the radio-sulfur is present in the aqueous layer as SO_4.

Since most of the ^{35}S is present in the neutron-bombarded carbon tetrachloride in a compound akin to $CSCl_2$, it is necessary to use heat as well as oxidizing agents (instead of bromine, chlorine or hydrogen peroxide is used as well) to extract completely the activity from the carbon tetrachloride. To obtain the highest specific activity (which was, when using the 60-in. Berkeley cyclotron (for yield, see page 40), about 1 millicurie active sulfur in 1–10 mg.), it was found advisable to remove all sulfur impurities from the carbon tetrachloride prior to irradiation.[10]

Active sulfur can also be concentrated by distillation of the irradiated carbon tetrachloride from inactive sulfur mixed with the chloride prior to bombardment, the active sulfur remaining with the carrier.[11] By this procedure, however, only about one-third of the active sulfur can be recovered.[12] At a high radiation level extensive decomposition of carbon tetrachloride takes place; therefore, potassium chloride is generally irradiated.

V. Production of Radiohalogens

Carrier-free ^{82}Br is prepared with fair yield in the cyclotron by the action of alpha rays on selenium:[13]

$$^{80}_{34}Se + {}^{4}_{2}\alpha = {}^{82}_{35}Br + {}^{1}_{1}H + {}^{1}_{0}n$$
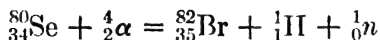
[10] M. D. Kamen, *Phys. Rev.*, **60**, 537 (1941).

[11] E. B. Andersen, *Z. physik. Chem.*, **B32**, 237 (1936).

[12] H. H. Voge, *J. Am. Chem. Soc.*, **61**, 1032 (1939).

[13] J. G. Hamilton, personal communication.

^{18}F (T = 112 min.) can be expected to be obtained by an analogous reaction by bombarding oxygen.

Practically weight-free radioiodide ^{131}I is obtained by bombardment of tellurium with swift deuterons (for yield, see page 41). The metal is dissolved in acid; the iodide is distilled off and collected in an alkaline solution which is then neutralized and brought to a physiological concentration. Very powerful samples of ^{131}I are obtained as fission products from the pile.

Radiohalogens are often produced by neutron capture of their stable isotopes. By the action of neutrons on ethyl iodide, for example, active free iodine is produced.[14] A large part of the active iodine formed does not react with the ethyl iodide and can be extracted, as was shown first by Szilard and Chalmers.[14] The extraction is greatly facilitated by the presence of traces of inactive iodine, which also will protect the radioiodine atoms formed from reaction with any impurities. The principal cause of the formation of radioiodine atoms by the action of neutrons on ethyl iodide or other iodine compounds is the recoil of the radioactive nucleus due to the emission of a γ-quantum, which supplies the energy necessary to break the carbon-iodine bond; active iodine atoms will then be formed. The energy of recoil, E_a, in electron volts is given by the equation:

$$E_a = 5.33 \times 10^{-10} E_\gamma^2/m$$

where E_γ is the energy of the γ-ray in electron volts, and m is the mass of the atom (H = 1).

The energies of the γ-rays for chlorine, bromine, and iodine, as well as the recoil energies and the energies of rupture of the carbon-halogen bond, E_{C-X}, are recorded in Table 1. It will be seen that the recoil energy is always much larger than the energy of rupture of the bond, so that every halogen atom which captures a neutron will be torn from the parent molecule and can react as a free radical possessing high excess energy.[15]

TABLE 1

Energies of γ-Ray, Recoil, and Bond Rupture[15]

Element	E_γ, e.v. $\times 10^6$	E_a, e.v.	E_{C-X}
Cl	6.2	585	3.3
Br	5.1	175	2.7
I	4.8	96	2.0

[14] L. Szilard and T. L. Chalmers, *Nature*, **134**, 462 (1934).
[15] C. S. Lu and S. Sugden, *J. Chem. Soc.*, **1939**, 1273.

The ejected radioactive halogen atom (denoted by an asterisk), formed by the action of neutrons on, $e.g.$, CH_3I, can, as long as it possesses high excess energy, replace monovalent atoms or atom groups of the surrounding medium.[16] Thus the formation of radioactive CH_2II^* from CH_3I, of $CHBr_2Br^*$ from CH_2Br_2, and of similar compounds was found to take place. Other radioactive organic compounds can be produced by irradiating halogen compounds dissolved in substances with which the high-energy atoms can react; when CH_3I dissolved in a great excess of benzene is irradiated with neutrons, $C_6H_5I^*$ is produced. Similarly, neutron irradiation of a saturated solution of I_2 or KI in acetic acid produces CH_3I^*, which can be removed by an air current passed through the solution. Here the high-speed halogen radical substitutes a COOH group in CH_3COOH.

Ejected radioactive halogen atoms ($e.g.$, I^*) that have lost their excess energy without having entered organic compounds react, in most cases, with the I_2 or HI molecules present:

or
$$I^* + I_2 \rightarrow I + II^*$$
$$I^* + HI \rightarrow I + HI^*$$

and then can be obtained by aqueous extraction.[14] The percentage of radioactive halogen entering organic compounds can be decreased, and the activity yield in the aqueous extract can thus be increased, by greatly diluting the irradiated halide with solvents containing no monovalent atoms or atom groups, $e.g.$, carbon disulfide.[16]

In addition to the high-speed reaction mentioned, organic radioactive halides are also produced by the combination, after slowing down, of radiohalogen with radicals that may have been formed by the impact of fast neutrons or fast atoms, or by γ-radiation. The extent of this after-reaction can be influenced by the presence of free halogen or aniline, or by pretreatment of the liquid to be irradiated.

To test the extent to which the presence of free halogens influenced the separation of radiohalides, parallel experiments were made by Lu and Sugden[15] in which the organic halide ($C_2H_4Br_2$) was submitted to the following pretreatments before irradiation with neutrons: (a) not treated, (b) shaken with aqueous sodium thiosulfate solution, and (c) mixed with 1–3% free halogens. In experiment (a), the extraction yield obtained was 51% of the bromine formed; in (b), the yield was 60%; and in (c), 69%. The best yield is thus obtained in the presence of some free halogens. In the cases of bromobenzene, n-butyl iodine,

[16] E. Glückauf and J. W. J. Fay, $J.$ $Chem.$ $Soc.$, **1936**, 390.

and iodobenzene, the yields were only slightly influenced by pretreatment of the compounds. Radiobromide is often produced by neutron bombardment of ethyl bromide.[17]

Aniline, when present during the irradiation, is found to have a very marked effect in increasing the fraction of activity that can be extracted by acids. The maximum effect is reached with about 4% aniline. In the case of irradiated chlorobenzene, the yield increased from 36 to 69% in the presence of 4% aniline. For bromobenzene the figures were 30 and 76%. The effect of aniline is presumably due to he reaction:

$$R + X^* + NH_2C_6H_5 \rightarrow C_6H_5 \cdot NH_2R^+ + X^{*-}$$
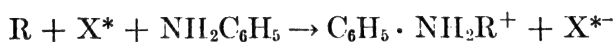
The X^{*-} ions thus formed have a much smaller reacting power than the X atoms. The best yields of extraction were obtained after neutron bombardment of the halides shown in Table 2.

TABLE 2

Yield of Extraction[15]

4% aniline plus	Extracting reagent (5%)	Extraction yield, %
CCl_4	HCl	88
$C_2H_4Br_2$	HCl	81
C_6H_5Br	HCl	76
C_6H_5Cl	HCl	69
C_4H_9I	$Na_2S_2O_3$	47

The following procedure was found to be very successful in preparing bromine with an activity of one millicurie and a weight of only about 10^{-6} mg. Bromobenzene containing 5% by volume of aniline was bombarded with neutrons. After the bombardment, the bromobenzene-aniline solution was shaken with water, and the aniline was removed from the water layer by extraction with ether. Good yields of ^{82}Br were also obtained in experiments in which acetylene tetrabromide was irradiated with slow neutrons.[18]

Radiochlorine of high activity can also be obtained by irradiating a solution of sodium chlorate or perchlorate or the solid salts of these compounds. If traces of chloride are added to the irradiated solution

[17] P. Süe, *J. chim. phys.*, **40**, 123 (1943).

[18] A. Roberts and J. W. Irvine, Jr., *Phys. Rev.*, **53**, 609 (1938).

and the chloride is precipitated as silver chloride, all radiochlorine is found in the precipitate.[19] By a similar method, radiobromine and radioiodine can also be concentrated.[18] Very high yields of concentrated halogens from neutron-irradiated organic halides were obtained using charcoal as adsorber.[20] Good yields of radioiodide were also obtained by shaking irradiated ethyl iodide with a solution of hydrogen sulfide.[21]

Radiofluorine can be prepared by the reactions $^{17}O(d,n)^{18}F$, ^{18}O $(d,2n)^{18}F$, or $^{20}Ne(d,\alpha)^{18}F$ with good yield. A few milliliters of distilled water is bombarded behind a 1-mm. aluminum window.[22] Radiocopper, present as an impurity, is removed by precipitation with hydrogen sulfide after adding a minute amount of copper fluoride as a carrier. An alternative procedure is the irradiation of lithium fluoride. Radio-arsenic of high concentration is obtained from irradiated cacodyl. Only a minor part of the activity is retained after extraction by the irradiated compound.[19] Starke has reported[23] a convenient way to obtain radio-arsenic. The irradiated cacodyl is shaken with magnesium oxide, which is then dissolved in hydrochloric acid. A minute amount of phosphate is added. By adding ammonia to the solution the phosphate and the radioarsenite are precipitated. To separate the radioarsenic from phosphorus, arsenic hydride is produced. Süe adds a few milligrams of sodium arsenite as a carrier; the arsenic is precipitated as a silver compound.[24]

VI. Concentration of Radioelements by Means of an Electric Field

As shown by Fay and Paneth,[25] it is also possible to collect radioactive isotopes in an electric field either in the gaseous or the liquid phase, a method previously used with much success in the collection of some of the natural radioactive isotopes — the "active deposit" of radium, thorium, and actinium. The active arsenic isotope, ^{76}As, formed through bombardment of gaseous arsine by neutrons, was found to attach itself to the positive and negative ions present in the gas and

[19] O. D'Agostino, *Gazz. chim. ital.*, **65**, 1071 (1935).

[20] O. Erbacher and K. Philipp, *Z. physik. Chem.*, **A176**, 169 (1936).

[21] P. Süe, *J. chim. phys.*, **40**, 123 (1943).

[22] J. F. Volker, H. C. Hodge, H. J. Wilson, and S. N. Van Voorhis, *J. Biol. Chem.*, **134**, 353 (1940).

[23] K. Starke, *Naturwissenschaften*, **28**, 631 (1940); *Physik. Z.*, **42**, 184 (1941).

[24] P. Süe, *J. chim. phys.*, **40**, 17 (1943).

[25] J. W. J. Fay and F. A. Paneth, *J. Chem. Soc.*, **1936**, 384.

to be deposited on electrodes of either sign.   On a copper spiral, 26% of the activity was found to be deposited in a negative, and 34% in a positive, field.   In the case of ethyl iodide, the iodine atoms which are activated and, at the same time, detached from the ethyl groups acquire by exchange processes the negative charge of iodine ions that are usually present in the liquid or, if not present, that can easily be added.   By electrolysis, therefore, the active isotope,[128]I, can be concentrated on a copper or silver anode.   By the same method, active bromine[26] can be collected from ethyl bromide and other organic bromides.   The concentration of ^{32}P by the action of an electric field is discussed on page 3.

VII.   Concentration of Radiometals

In contrast to the separation of radiohalogens, radioarsenic, and some other radioelements from their inactive isotopes, the concentration of many of the radiometals presents great difficulties.[27]   For gold, a restricted concentration of the radioactive atoms was obtained[28] by irradiating gold thiosulfate with neutrons and shaking the irradiated compound with mercury.   The gold obtained after distilling off the mercury was found to be more active than the gold of the irradiated compound.   Another method of separation is the following: A colloidal gold solution is bombarded with neutrons and the fraction precipitated during irradiation is collected.   This fraction is found to be about nine times more active than the nonprecipitated gold present in the colloidal solution.

Steigman[29] points out that a metal complex compound whose optically active isomers do not racemize can be used to concentrate radioactivity produced by neutron capture.   A solution of about 1 g. $Rh(en)_3(NO_3)_2$, where "en" denotes ethylenediamine, was irradiated with slow neutrons; 10 to 20 mg. of "carrier," rhodium chloride, zinc dust and hydrochloric acid were then added.   The "free" metal ion was reduced to the metal and filtered off with the excess of zinc dust. The ratio of concentrate activity to activity of the complex salt was found to be 150.   Somewhat smaller ratios (56, 44, and 10) were found

[26] P. C. Capron, G. Stokking, and M. van Meerssche, *Nature*, **157**, 806 (1946).

[27] E. Amaldi, O. D'Agostino, E. Fermi, B. Pontecorvo, F. Rasetti, and E. Segrè, *Ricerca sci.*, **2**, No. 11–12 (1934).

[28] V. Majer, *Naturwissenschaften*, **25**, 252 (1937).

[29] J. Steigman, *Phys. Rev.*, **59**, 498 (1941).

when concentrating radioiridium, radioplatinum, and radiocobalt, respectively, by an analogous method.

Radiomanganese can be concentrated easily. When an irradiated aqueous solution of potassium permanganate is filtered, most of the activity is found on the paper in a thin deposit of manganese dioxide.[30] The neutron capture recoil causes one or two oxygen ions to be ejected by the permanganate ion.[31] Attempts to concentrate radiomanganese from irradiated acetylacetonate or benzoylacetonate of manganese failed.[32] The electrochemical separation of radioelements is discussed in a recent book of Haïssinsky.[33]

VIII. Separation of Nuclear Isomers

Some isotopes, ^{80}Br for example, have nuclear isomers. ^{80}Br when formed by the action of neutrons or deuterons on ^{79}Br is first in a higher-nuclear-energy state. It releases the excess nuclear energy in the form of γ radiation with a half-life of 4.4 hours and is converted to ^{80}Br of lower isomeric state. These ^{80}Br atoms of lower isomeric state then decay with a half-life of 18 minutes, emitting γ-particles, and are converted to ^{80}Br. The energy released during the transition from an upper to a lower isomeric state of atomic nucleus can be used to effect a chemical separation [34,35] of the two ^{80}Br isomers in a similar way to that in which radioactive ^{80}Br is separated from stable ^{79}Br and ^{81}Br. In this case, however, unlike that discussed in a preceding section, it is not the recoil energy which is responsible for the breaking of the chemical bond producing the separation. When the ^{80}Br atom passes through the isomeric transition, an internal conversion electron is emitted. As a result of this emission, the ^{80}Br atom acquires a recoil energy. This energy, however, in the majority of cases is only about 5.6 kg.-cal. (2.4 × 10^{-1} e.v.) per mole, which is too small to account for the bond rupture observed with bromine compounds. The bond rupture was found, in such cases, to be a consequence of the high state of excitation of the atom out of which the electron has been ejected.[34]

The radioactive isotopes ^{127}Te and ^{129}Te also undergo isomeric

[30] E. Fermi, E. Amaldi, et al., Proc. Roy. Soc. London, **A149**, 522 (1935).

[31] W. F. Libby, J. Am. Chem. Soc., **62**, 1930 (1940).

[32] P. Süe and T. Yuasa, J. chim. phys., **41**, 160 (1944).

[33] M. Haïssinsky, Electrochimie des substances radioactives et des solutions extrêmement diluées. Masson, Paris, 1946.

[34] E. Segrè, R. S. Halford, and G. T. Seaborg, Phys. Rev., **55**, 321 (1939).

[35] D. De Vault and W. F. Libby, Phys. Rev., **55**, 322 (1939).

transitions and, as an effect of these transitions, telluric acid is reduced to tellurous acid with approximately 100% yield in aqueous solution at room temperature or even when frozen to the temperature of liquid air.[36] The tellurium atom is excited after K-shell conversion to the extent of 740,000 kg.-cal. per mole, and after L-shell conversion to 100,000 kg.-cal. per mole. During the electronic rearrangement which follows, the telluric acid molecule has ample opportunity to dissociate, a process preceded by the formation of a tellurous acid molecule. This technique was also applied in the separation of the selenium isotopes. No rupture of the $Zn(C_2H_5)$ bond, however, takes place during isomeric transition of ^{69}Zn.[37] Isotopes which display the phenomenon of nuclear isomerism coupled with internal conversion must be used with great precaution as tracers.

IX. Preparation of Tritium ($_3$H)

Tritium ($T = 31$ years) is obtained by subjecting a beryllium target to deuteron bombardment in the cyclotron.[38] The tritium is collected in the form of HTO by dissolving the beryllium target in 6 ml. hydrochloric acid in a vacuum system, passing the evolved hydrogen and tritium over copper oxide at 550° C. and collecting the water in a liquid air trap. An activity of 2×10^7 counts per second was obtained in 1 ml. tritiated water prepared from a beryllium target, $1.9 \times 1.9 \times 0.08$ cm., which had received 20 hours of bombardment in a 25-m.e.v. deuteron beam.[39] When very strong neutron sources are available, lithium is bombarded and splits into ^3_1H and ^4_2He, thus supplying tritium.

X. Production of Tracer Carbon

While strong sources of ^{11}C can easily be obtained by deuteron or proton bombardment of boron (for yield, see page 40), the preparation of strongly active ^{14}C samples is only possible by using the copious source of neutrons supplied by the pile. The yield of the three reactions:

$$^{13}_6C + ^2_1D \rightarrow ^{14}_6C + ^1_1H \tag{a}$$

$$^{14}_7N + ^1_0n \rightarrow ^{14}_6C + ^1_1H \tag{b}$$

$$^{11}_5B + ^4_2He \rightarrow ^{14}_6C + ^1_1H \tag{c}$$

[36] G. T. Seaborg, J. J. Livingood, and J. W. Kennedy, *Phys. Rev.*, **57**, 363 (1940).

[37] J. J. Livingood and G. T. Seaborg, *Phys. Rev.*, **55**, 457 (1939).

[38] R. D. O'Neal and M. Goldhaber, *Phys. Rev.*, **57**, 1086 (1940).

[39] N. Pace, L. Kline, H. K. Schachman, and M. Harfenist, *J. Biol. Chem.*, **168**, 459 (1947).

leading to the formation of ^{14}C is very low and amounts only to a very small fraction of the yield obtained in the preparation of ^{11}C.

Ruben and Kamen[40] bombarded ten gallons of saturated ammonium nitrate with neutrons produced by the cyclotron, thus making use of the capture of slow neutrons by the nitrogen nuclei to produce ^{14}C. After exposure of the solution for six months to the effect of neutrons, samples emitting 10^5 counts per minute were obtained. Pile-produced radioactive tracers are obtained according to equation b on page 14, the bombardment of ammonium nitrate or other nitrogen-containing compounds supplying the ^{14}C. Barium carbonate having as high as 8% ^{14}C has been prepared, while 2% preparations are now common. Norris and Snell[41] describe the use of a circulating solution of ammonium nitrate when preparing ^{14}C with a pile. Radiocarbon is also produced from atmospheric nitrogen by the reaction ^{14}N(n,p)^{14}C under the action of neutrons produced by cosmic radiation. Due to this fact, biomethane (of recent origin), in contrast to ("old") petromethane, shows a slight radioactivity.[42]

XI. Production of Radiosodium and Radiopotassium

^{24}Na and ^{42}K are prepared by deuteron or neutron bombardment of sodium chloride or potassium chloride, respectively (for yield, see page 40). Since all atoms present in the pure element sodium can contribute to the formation of ^{24}Na, while only 7% of the potassium atoms (those having the mass number 41) can form ^{42}K, much stronger preparations of ^{24}Na than of ^{42}K are obtained (see page 40). ^{24}Na — mostly present in irradiated potassium — can, after the addition of some carrier sodium, be removed by precipitation of the potassium with perchloric acid and ethyl alcohol.

Carrier-free ^{22}Na is obtained from magnesium by a (d, α) reaction with poor yield. Carrier-free ^{42}K was prepared by Hamilton[43] by the action of 40-m.e.v. alpha particles on argon gas percolating through a gold vessel. The ^{42}K was collected on the walls of the vessel and in the glass wool plug through which the cooled irradiated argon streams out. No suitable reaction is known which would permit its production by the uranium pile. The "natural" radioactivity of potassium is usually

[40] S. Ruben and M. D. Kamen, *J. Applied Phys.*, **12**, 31 (1941).

[41] L. D. Norris and A. H. Snell, *Science*, **105**, 265 (1947).

[42] E. C. Anderson, W. F. Libby, S. Weinhouse, A. F. Reid, A. D. Kirshenbaum, and A. V. Grosse, *Science*, **105**, 2735 (1947).

[43] J. G. Hamilton, personal communication.

negligible compared with the activity of ^{42}K. 36 β-particles per minute are emitted from 1-cm.^{2} surface of a thick potassium layer.

XII. Production of Tracer Calcium and Strontium

Radiocalcium is prepared by the bombardment of calcium metal with deuterons (for yield, see page 40) in the cyclotron according to the following nuclear reaction:

$$^{40,44}Ca + {}^2_1H \rightarrow {}^{41,45}Ca + {}^1_1H$$
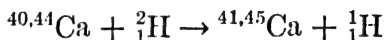
After allowing about six weeks to elapse so that the ^{41}Ca and ^{44}Sc may disintegrate to negligible amounts, the metal containing ^{45}Ca is dissolved in dilute hydrochloric acid. The calcium is precipitated as oxalate, dissolved, and reprecipitated. The oxalate is converted to calcium carbonate by ignition. This is dissolved in lactic acid, for example, which can be conveniently fed.[44] A very much higher yield can be expected to be obtained by the action of neutrons on scandium:

$$^{45}_{21}Sc + {}^1_0n \rightarrow {}^{45}_{20}Ca + {}^1_1H$$
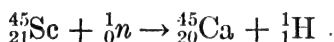
^{89}Sr ($T = 55$ days) is obtained by the nuclear reaction:

$$^{88}_{38}Sr + {}^2_1H \rightarrow {}^{89}_{38}Sr + {}^1_1H$$
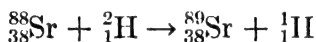
Since there is 82% ^{88}Sr in ordinary strontium, good yield is obtained (see page 40). Powerful ^{89}Sr preparations containing ^{90}Sr ($T = 5$ years) are supplied as fission products by the pile, which also produces ^{89}Sr using the Sr (n,γ) reaction.

XIII. Preparation of Radioiron and Radiomanganese

A. PRODUCTION OF ^{59}Fe

^{59}Fe of almost negligible activity is obtained when bombarding iron in the 60-in. cyclotron (for yield, see page 40); simultaneously, however, radiocobalt of pronounced activity is formed. A practical method for obtaining ^{59}Fe is neutron bombardment of cobalt according to the equation $^{59}_{27}Co + {}^1_0n \rightarrow {}^{59}_{26}Fe + {}^1_1H$. ^{59}Fe is recovered in the following manner. To 50 ml. of the irradiated solution of $CoCl_2 \cdot 6H_2O$ which was exposed for several months to neutrons in the cyclotron, 50 mg. uranium as uranyl chloride is added as carrier. Precipitation of iron and uranium is obtained by adding carbon-dioxide-free ammonia in great excess to obtain all cobalt remaining in solution. The precipitate

[44] W. W. Campbell and D. M. Greenberg, *Proc. Natl. Acad. Sci. U. S.*, **26**, 176 (1940).

is washed with 1% ammonium chloride in 1 N ammonium hydroxide and then dissolved in warm dilute hydrochloric acid. The procedure described, solution following precipitation, is repeated until the precipitate is entirely free of cobalt. When five liters of cobalt chloride solution are irradiated, the procedure described is carried out with nine more aliquots; the cobalt-free hydrochloric acid solutions are then united.

To separate the iron from the uranium the following steps are taken: The solution is evaporated to a volume of 25 ml. and a hydrochloric acid content corresponding to 8 N. In a separating funnel the solution is shaken with 25 ml. isopropyl ether. The extraction is repeated until the extract gives no iron test with potassium thiocyanide. The ether fractions are united; the ether is boiled off, 10 ml. water added and the hot solution precipitated with 10% ammonium carbonate in 6 N ammonium hydroxide. The solution is centrifuged and the iron precipitate dissolved in hydrochloric acid. The solution is precipitated again, and again dissolved in hydrochloric acid. When precipitated a third time, the ferric hydroxide obtained is dissolved in a calculated amount of warm saturated citric acid.[45] Iron citrate mainly is used in tracer experiments. As the large amounts of cobalt chloride irradiated contain some iron as impurity, the ^{59}Fe obtained contains several milligrams of iron.

B. PRODUCTION OF ^{55}Fe

^{55}Fe can be prepared with fairly good yield (page 40) by the reaction:

$$^{55}_{25}\text{Mn} + ^{2}_{1}\text{D} \rightarrow ^{55}_{26}\text{Fe} + 2^{1}_{0}n$$
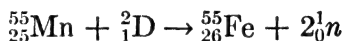
The irradiated manganese is dissolved in boiling dilute hydrochloric acid; 10 mg. iron as ferric chloride is added as carrier. After adding 25 mg. copper as cupric chloride the 0.3 N hydrochloric acid solution is heated to boiling. Passing hydrogen sulfide through the solution copper, lead, tin, antimony and bismuth precipitate, while the filtrate contains iron as well as manganese and perhaps zinc. The filtrate is brought to 50 ml. and 5 ml. of 30% hydrogen peroxide is added. When boiling the solution, iron is oxidized. By making the solution alkaline with ammonium hydroxide the iron is precipitated as ferric hydroxide. Some manganese will also precipitate; but this should be disregarded, since this step provides a means only of separating the iron from any

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[45] R. C. Lilly, personal communication.

large amounts of salts which may be present to interfere with the clear extraction. The hydroxide precipitate is dissolved in a small amount of warm 9 N hydrochloric acid resulting in a volume of approximately 10–25 ml. of 8 N hydrochloric acid concentration. The solution is stirred for 5–10 minutes after the addition of 15–20 ml. isopropyl ether. The extraction is repeated three times. After boiling off the ether, the ferric chloride is taken up with a few milliliters of water, ammonium hydroxide is added, and ferric hydroxide obtained centrifuged. The precipitate is dissolved in hydrochloric acid and precipitated, this precipitate being dissolved in a hot, saturated solution of citric acid; the solution is then neutralized with ammonium hydroxide.[45]

Radiomanganese is often produced from iron by deuterium bombardment according to the reactions: $^{56}\text{Fe}(d,a)^{54}\text{Mn}$, and $^{52}\text{Cr}(d,2n)^{52}\text{Mn}$, respectively.

XIV.   Preparation of Radiocopper

Radiocopper can be prepared from zinc irradiated with fast neutrons.[46] When dissolving the irradiated zinc in acid, the last nondissolved residue contains most of the radiocopper. Another procedure is based on the addition of inactive copper ion to the solution of irradiated zinc, followed by electrolytic displacement of the labeled copper on a platinum electrode. The application of a high-speed rotating cathode in the separation of radiocopper was found to be very useful.[47] ^{64}Cu can also be prepared by the action of protons on nickel[48] according to the equation:

$$^{64}_{28}\text{Ni} + ^{1}_{1}\text{H} \rightarrow ^{64}_{29}\text{Cu} + ^{1}_{0}n$$
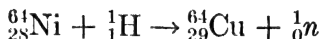
Very strong ^{64}Cu preparations are obtained by deuterium bombardment of a copper foil in the cyclotron (see page 40).

The electrochemical separation of radioelements is discussed in the recent book of Haïssinsky.[33]

XV.   Preparation of Radiozinc

By a $(d,2n)$ process ^{65}Zn is obtained in good yield from an irradiated copper target (see page 40). The copper is dissolved in 6 N nitric acid. After driving off the excess acid, the cupric nitrate is redissolved

[46] E. Fermi, E. Amaldi, et al., Proc. Roy. Soc. London, **A146**, 83 (1934).

[47] M. Haïssinsky, Nature, **136**, 141 (1935).

[48] L. N. Ridenour, L. A. Delsasso, M. G. White, and R. Sherr, Phys. Rev., **53**, 770 (1938).

in a small amount of warm water, adding 2 ml. concentrated nitric acid and 2 ml. concentrated sulfuric acid. To this solution 5 mg. zinc as zinc sulfate is added to serve as carrier for ^{65}Zn. The solution is transferred to a platinum evaporating dish and the copper is precipitated electrolytically. The copper-free solution is brought to 5 ml., 2–5 ml. 30% hydrogen peroxide is added, and the solution is boiled until all hydrogen peroxide is destroyed. The solution is made strongly ammoniacal and boiled for a few minutes to obtain precipitation of ferric hydroxide which may be present.

The filtrate is made 0.3 N with hydrochloric acid and hydrogen sulfide passed in to precipitate copper possibly still present. After driving off excess hydrogen sulfide by adding ammonium hydroxide a pH of 2–3 is obtained. Passing hydrogen sulfide through the solution precipitates zinc sulfide. The washed precipitate is dissolved in hydrochloric acid and reprecipitated; the precipitate is dissolved in hydrochloric acid and after evaporating the filtrate to dryness the zinc chloride is taken up in water.[45] If zinc of very high specific activity is desired, the following procedure is used.[43] The copper target is dissolved in concentrated nitric acid; copper is removed from the nitric acid solution by a threefold precipitation as copper sulfide in 0.25 N hydrochloric acid and 2 N ammonium chloride. The filtrate and precipitate are treated in the same manner as above.

XVI. Production of Radioactive Inert Gases

^{37}A, ^{79,81}Kr, and ^{127}Xe are prepared by the $(d,2n)$ reaction (bombardment with deuterons) on the chloride, bromide, or iodide, respectively, of potassium. Some of the gas produced being occluded in the salt, the latter must be molten to obtain a quantitative recovery of the gas produced. ^{41}A is prepared by deuteron bombardment of argon gas.[49]

[49] J. G. Hamilton and C. A. Tobias, personal communication.

Radioactive Isotopes of Possible Interest in Tracer Work

Radioactive tracers are prepared under the action of neutrons, protons, deuterons, or alpha particles on stable isotopes. A complete list of all known isotopes compiled by Segrè appears at the end of the book.[1]

The pile is a most copious source of slow neutrons, which are utilized in the preparation of radioactive isotopes. Furthermore, numerous radioactive substances are formed in the pile as fission products. The radioactive isotopes obtained by making use of the pile are listed in Table 3 published by the Atomic Energy Commission. This table includes most of the radioisotopes of importance in biological investigations, such as ^{3}H, ^{14}C, ^{24}Na, ^{32}P, ^{35}S, ^{36}Cl, ^{42}K, ^{45}Ca, ^{55}Fe, ^{59}Fe, ^{64}Cu, ^{65}Zn, ^{82}Br, ^{89}Sr, and ^{131}I. ^{14}C, for example, though discovered[2] by making use of the Berkeley cyclotron, can be produced in larger quantities with the pile only. To produce a millicurie of ^{14}C by normal cyclotron methods would require at least five efficient cyclotrons running continuously for a year (2000 total days bombardment) at a final cost of around a million dollars. With the pile, on the other hand, hundreds of millicuries of ^{14}C will readily become available. Aebersold[3] states that in the first four months of distribution, the Manhattan Project had already distributed about 50 millicuries of ^{14}C, which according to the above normal cyclotron production figures would require 250 cyclotron years. By special bombardment procedures, the cyclotron efficiency of ^{14}C production could probably be increased by a factor of 10 or even 100. Nevertheless the cyclotron production would be laborious, limited, and costly (probably not below several thousand dollars per millicurie). The initial production cost of ^{14}C using the pile was around $400 per millicurie, but increased production lowered this cost to $50.

[1] Courtesy Dr. E. Segrè and Addison-Wesley Press Inc., Cambridge, Mass.
[2] S. Ruben and M. D. Kamen, *Phys. Rev.*, **59**, 349 (1941).
[3] P. C. Aebersold, personal communication.

TABLE 3.   Radioisotopes Supplied by the Atomic Energy Commission[a]

A. Separated Radioisotopes

Item No.[a]	Element	Price, per mc.	Half-life	β-Radiation	γ-Radiation	Comments
S-1	^{14}C	$50	5100 y.	0.154 m.e.v.		Shipped as $BaCO_3$ in which 3–5% of C atoms are radioactive. 1 mc. ^{14}C will be carried in 100–175 mg. $BaCO_3$.
S-2	^{131}I	$1.70	8.0 d.	0.6 m.e.v.	0.367 and 0.080 m.e.v.	Carrier-free in neutral or basic weak sol. containing 0.3–1.0 mc./ml. Chem. anal. furnished with each shipment. Material will meet the following specifications: Concn., 0.5 mc./ml. pH, 7–9 Total solids, 1 mg./ml. Te inactive, 0.1 mg./ml. Te active, $< 1 \times 10^{-4}$ mc./ml.
S-3	^{32}P	$1.10	14.3 d.	1.69 m.e.v.		Phosphate ion probably in form of Na_2HPO_4. Chem. anal. furnished with each shipment. Material will meet the following specifications: Concn., > 0.5 mc./ml. pH, 7–9 Total solids, < 10 mg./ml. Nonvolatile matter, < 5 mg./ml. P (inert), approx. 0.025 mg./mc. added[b] Cl, < 5 mg./ml. Fe, Ni, Al } Content such that no ppt. is present at pH 7–9
S-4	^{35}S	$2.40 supplied as H_2SO_4; $6.00 supplied as Na_2S*	87.1 d.	0.17 m.e.v.		1.53 mc./ml., 0.1 mg. SO_4/ml. 1.96 mc./ml., 0.5 mg. SO_4/ml.

[a] From Catalog and Price List No. 2, effective March 1, 1947, and the issue revised September, 1947; distributed by U. S. Atomic Energy Commission, Oak Ridge, Tenn.

[b] Carrier-free material is available if desired.

* Carrier added.

TABLE 3 (*continued*)

B. Fission Products[c]

Catalog item no.	Radioisotope	Price, per mc.	Catalog item no.	Radioisotope	Price, per mc.
I	(^{95}Zr) (^{95}Cb) } d	$0.67	I-A	^{95}Cb	$23.09
II	^{91}Y	1.15	II-A	(^{103}Ru) (^{106}Ru) } d	23.09
III	(^{141}Ce) (^{144}Ce) (^{144}Pr) } d	1.35			
IV	^{140}Ba ^{140}La } d				
V	(^{89}Sr) (^{90}Sr) (^{90}Y) } d	1.35			
VI	(^{143}Pr) (^{147}Nd) } d $(^{147}61)$ (^{155}Eu) (^{156}Eu) } d	12.51	VI-A	^{143}Pr	72.16
			VI-B	^{147}Nd	72.16
			VI-C	$^{147}61$	14.43
VII	^{137}Cs	134.70			
VIII	(^{103}Ru) (^{106}Ru) (^{106}Rh) } d (^{127}Te) (^{129}Te)	6.74	VIII-A	(^{103}Ru) (^{106}Ru) } d	23.00
			VIII-B	(^{127}Te) (^{129}Te) } d	23.09

[c] Fission products are not routinely available — any order placed is subject to cancellation due to frequent changes in operating schedules. Materials are shipped in weak acid solution in which no carrier has been added. No nonvolatile solids are present. More complete data on the nuclear properties of fission products are given in *J. Am. Chem. Soc.*, **68**, 2411–2442 (1946), and *Rev. Modern Phys.*, **18**, 513–544 (1946). See also Catalogue and Price List No. 2, revised September, 1947; some data from revised list included here.

[d] Mixtures.

TABLE 3 (*continued*)

C. Irradiated Units[e]

Catalog item no.	Radioisotopes in unit Alphabetical list	Others present	Also see unit no.	Half-life[f]
3A	Antimony 122		3B	2.8 d.
		^{124}Sb		60 d.
3B	Antimony 124		3A	60 d.
		^{122}Sb		2.8 d.
3C	Antimony 125			2.7 y.
		^{113}Sn		100 d.
		^{121}Sn		62 hr.
		^{123}Sn		10 d.
		^{125}Sn		9 mo.
		(parent of ^{125}Sb)		
4	Argon 37			34 d.
		Ca 45		180 d.
5A	Arsenic 76			26.8 hr.
5B	Arsenic 77		5C	40 hr.
		^{71}Ge		$\begin{cases} 11 \text{ d.} \\ 40 \text{ hr.} \end{cases}$
		^{77}Ge		12 hr.
		(parent of ^{77}As)		
5C	Arsenic 77	1/10 of Unit 5B		
7	Barium 131 (parent of ^{131}Cs)			12 d.
		^{131}Cs		10.2 d.
		^{14}C		5100 y.
9A	Bismuth 210		9B	5 d.
		^{210}Po		140 d.
9B	Bismuth 210	1/10 of Unit 9A		

[e] An irradiated unit is a specified quantity of target material that has been sealed in an aluminum case and irradiated in the pile. The unit is shipped without chemical processing. Several isotopes are present in more than one unit; reference to all units in which an isotope is found in significant quantities is given opposite the name of the isotope. Requests for smaller quantities per unit may be approved but no reduction in price will be made since production costs remain the same. In those few cases where the value of the target material is appreciable, units are available in two sizes, the standard

TABLE 3 *(continued)*

C. Irradiated Units[e] *(continued)*

Radiation (m.e.v.)		Target material		Estimated quantity (mc.)	Mc./gm. element (est.)[f]	Price per unit
Beta	Gamma	Compound	Grams			
1.36, 1.94	0.57	Sb	0.20	50	250	$12.00
0.53, 2.25	1.72			1	5	
0.53, 2.25	1.72	Sb	0.20	4	20	33.00
1.36, 1.94	0.57			55	275	
0.8, 0.3	Present	Sn	6.2	1.0	CF	33.00
K, e^-	0.085			1.0	0.16	
0.8	None			?	?	
2.6	Present			?	?	
$\sim$2.2	$\sim$0.74			?	?	
K	None	$CaCO_3$	25	0.2	CF	33.00
0.25	None			0.8	0.1	
1.1, 1.7, 2.7	0.57, 1.25	As_2O_3	0.06	25.0	560	12.00
0.8	None	GeO_2	1.5	0.70	CF	52.00
$\begin{cases} 0.6\ \beta^+ \\ 1.2\ \beta^+ \end{cases}$	0.6			10	10	
1.9				0.7	0.7	.
						13.00
K, e^-	1.2 (weak)	$Bi(NO_3)_2$	44	6.0	0.26	33.00
K	None			9.0	CF	
0.154	None			0.02	CF	
1.17	None	Bi	24.5	10	0.4	25.00
5.298 α, e^-	0.8 (weak)			0.3[g]	CF	
						12.00

size and a 1/10 size at a reduced price. Irradiation units are offered with the understanding that because of variations in production setup, activities may differ 50% from the quantities estimated. See revised price list (No. 2) for additional data and additional isotopes.

[f] d. =days, hr. =hours, mo. =months, y. =years, CF = carrier-free.

[g] Maximum quantity 25 days after removal from pile.

TABLE 3 (*continued*)

C. Irradiated Units[e] (*continued*)

Catalog item no.	Radioisotopes in unit Alphabetical list	Others present	Also see unit no.	Half-life[f]
11	Bromine 82			34 hr.
		^{42}K		12.4 hr.
	Cadmium 109		12A, 12B	
12A	Cadmium 115		12B	2.33 d.
		^{109}Cd		300 d.
		^{115}Cd		43 d.
12B	Cadmium 115		12A	43 d.
		^{109}Cd		300 d.
		^{115}Cd		2.33 d.
13	Calcium 45		4, 69A	180 d.
		^{37}A		34 d.
	Carbon 14		7	
15	Cerium 141			28 d.
		^{143}Ce (parent of ^{143}Pr)		33 hr.
		^{143}Pr		13.8 d.
	Cerium 143		15	
	Cesium 131		7	
16	Cesium 134			2 y.
17	Chlorine 36			10^6 y.
		^{42}K		12.4 hr.
		^{35}S		87.1 d.
		^{32}P		14.3 d.

[e] An irradiated unit is a specified quantity of target material that has been sealed in an aluminum case and irradiated in the pile. The unit is shipped without chemical processing. Several isotopes are present in more than one unit; reference to all units in which an isotope is found in significant quantities is given opposite the name of the isotope. Requests for smaller quantities per unit may be approved but no reduction in price will be made since production costs remain the same. In those few cases

TABLE 3 (continued)

C. Irradiated Units[e] (continued)

Radiation (m.e.v.)		Target material		Estimated quantity (mc.)	Mc./gm. element (est.)[f]	Price per unit
Beta	Gamma	Compound	Grams			
0.465	(0.547, 0.787, 1.35)	KBr	0.9	70	120	$12.00
3.58 (75%), 2.07 (25%)	1.51 (25%)			3	10	
1.13, 0.6	0.65	Cd	1	20	20	12.00
K	None			?	?	
1.5	~0.5			0.25	0.25	
. 1.85	~0.5	Cd	1	1	1	33.00
K	None			?	?	
1.13, 0.6	0.65			20	20	
0.250	None	CaCO₃	0.25	0.8	0.1	33.00
K	None			0.2	CF	

(Also available in separated form)

Radiation (m.e.v.)		Target material		Estimated quantity (mc.)	Mc./gm. element (est.)[f]	Price per unit
0.55	0.21	CeO₂	0.83	50	75	33.00
1.33	0.5			12	18	
0.95	None			3	CF	
0.645	0.8	Cs₂CO₃	0.6	20	40	34.00
0.66	None	KCl	25	0.005	0.0005	33.00
3.58 (75%)	1.51 (25%)			200	15	
2.07 (25%)						
0.17	None			100	CF	
1.69	None			0.01	CF	

where the value of the target material is appreciable, units are available in two sizes, the standard size and a 1/10 size, at a reduced price. Irradiation units are offered with the understanding that because of variations in production setup, activities may differ 50% from the quantities estimated. See revised price list (No. 2) for additional data and additional isotopes.

[f] d. =days, hr. =hours, mo. =months, y. =years, CF =carrier-free.

TABLE 3 *(continued)*

C. Irradiated Units[e] *(continued)*

Catalog item no.	Radioisotopes in unit Alphabetical list	Others present	Also see unit no.	Half-life[f]
18	Chromium 51			26.5 d.
19	Cobalt 60			5.3 y.
21	Copper 64			12.8 hr.
24A	Europium 154			5–8 y.
		^{152}Eu		9.2 hr.
24B[h]	Europium 155		68	2 y.
		^{155}Sm (parent of ^{155}Eu)		25 mo.
		^{153}Sm		47 hr.
28	Gallium 72			14.1 hr.
	Germanium 71		5B, 5C	
	Germanium 77		5B, 5C	
30A	Gold 198			2.7 d.
30B	Gold 199			3.3 d.
		^{197}Pt		18 h., 3.3 d.
		^{199}Pt (parent of ^{199}Au)		31 min.
31[h]	Hafnium 181			46 d.
35[h]	Element (61) 147			4 y.
		^{149}61		2 d.
		^{147}Nd		11 d.
		^{149}Nd (parent of ^{149}61)		47 hr.

[e] An irradiated unit is a specified quantity of target material that has been sealed in an aluminum case and irradiated in the pile. The unit is shipped without chemical processing. Several isotopes are present in more than one unit; reference to all units in which an isotope is found in significant quantities is given opposite the name of the isotope. Requests for smaller quantities per unit may be approved but no reduction in price will be made since production costs remain the same. In those few cases where the value of the target material is appreciable, units are available in two sizes, the standard

TABLE 3 *(continued)*

C. Irradiated Units[e] *(continued)*

| Radiation (m.e.v.) | | Target material | | Estimated quantity (mc.) | Mc./gm. element (est.)[f] | Price per unit |
Beta	Gamma	Compound	Grams			
K	0.32	Cr	1.1	50	50	$34.00
0.3	1.1, 1.3	Co₃O₄	0.9	20	30	33.00
0.58 β^- 0.66 β^+ K	1,2 (weak)	Cu	0.32	100	300	12.00
{ 0.34 (50%) 0.82 (50%) 1.61	1.4 *et al.*	Eu₂O₃	0.25	40	180 1/40 unit	2168.00 84.00
0.3 0.73	0.084 0.1, 0.57	Sm₂O₃	0.01	0.01 16	CF 2000	31.00
3.1, 0.8	0.84, 2.25	Ga(NO₃)₃	0.55	25	170	21.00
0.97	0.44	Au	0.016	80	5000	12.00
1.01 0.72 1.8	0.45 Present	Pt	0.5	10 7	CF 14	12.00
0.8	0.5	HfO₂	0.9	50	60	31.00
0.223 ? 0.4, 0.9 0.78, 1.2	None ? 0.55 0.25	Nd₂O₃	0.01	0.001 0.1 0.03 ?	CF CF 3.5	31.00

size and a 1/10 size, at a reduced price. Irradiation units are offered with the understanding that because of variations in production setup, activities may differ 50% from the quantities estimated. See revised price list (No. 2) for additional data and additional isotopes.

[f] d. =days, hr. =hours, mo. =months, y. =years, CF =carrier-free.

[h] Target material to be furnished by requester.

TABLE 3 (*continued*)

C. Irradiated Units[e] (*continued*)

Catalog item no.	Radioisotopes in units Alphabetical list	Others present	Also see unit no.	Half-life[f]
	Element (61) 149		**35**	
36A	Indium 114		36B	48 d.
36B	Indium 114		1/10 of Unit 36A	
37	Iodine 131			8 d.
		^{127}Te		90 d.
		^{129}Te		32 d.
		^{131}Te (parent of ^{131}I)		30 hr.
	Iodine 131	(Also available in separated form)		
38A	Iridium 192		38B	75 d.
		^{194}Ir		19 hr.
38B	Iridium 194		38A	19 hr.
		^{192}Ir		75 d.
	Iron 55		39	
39	Iron 59			44 d.
		^{55}Fe		4 y.
41	Lanthanum 140			40 hr.
47A	Mercury 197		47B	$\begin{cases} 64 \text{ hr.} \\ 25 \text{ hr.} \end{cases}$
		^{203,205}Hg		51.5 d.
47B	Mercury 203, 205		47A	51.5 d.
		^{197}Hg		$\begin{cases} 64 \text{ hr.} \\ 25 \text{ hr.} \end{cases}$

[e] An irradiated unit is a specified quantity of target material that has been sealed in an aluminum case and irradiated in the pile. The unit is shipped without chemical processing. Several isotopes are present in more than one unit; reference to all units in which an isotope is found in significant quantities is given opposite the name of the isotope. Requests for smaller quantities per unit may be approved but no reduction in price will be made since production costs remain the same. In those few cases

TABLE 3 (*continued*)

C. Irradiated Units[e] (*continued*)

Radiation (m.e.v.)		Target material		Estimated quantity (mc.)	Mc./gm. element (est.)[f]	Price per unit
Beta	Gamma	Compound	Grams			
IT[i], e^-, 1.95	0.19	In(NO$_3$)$_3$	0.36	10	70	$89.00
						36.00
0.6	0.367, 0.080	Te	50	130	CF	33.00
IT, e^-, 0.7	0.086			10	0.20	
9.3 h isomer						
IT, e^-, 1.8	0.102, 0.3, 0.8			8	0.16	
70 m isomer						
IT	0.177			10	0.20	
0.59	0.2–0.6	IrO$_2$	0.22	40	210	35.00
2.07	0.38, 1.65			200	1050	
2.07	0.38, 1.65	IrO$_2$	0.05	40	930	12.00
0.59	0.2–0.6			3	70	
0.26, 0.46	1.1, 1.3	Fe	17	1.0	0.06	33.00
K	0.07			0.9	0.05	
1.4 (90%)	1.63 complex	La$_2$O$_3$	0.09	40	525	12.00
2.2 (10%)						
$\begin{cases} K, e^- \\ K, e^- \end{cases}$	0.075	HgO	14	95	8	12.00
	0.13, 0.16					
0.3	0.28			40	3	
0.3	0.28	HgO	14	135	11	33.00
K, e^-	0.075			110	9	
K, e^-	0.13, 0.16					

where the value of the target material is appreciable, units are available in two sizes, the standard size and a 1/10 size, at a reduced price. Irradiation units are offered with the understanding that because of variations in production setup, activities may differ 50% from the quantities estimated. See revised price list (No. 2) for additional data and additional isotopes.

f d. =days, hr. =hours, mo. =months, y =years, CF =carrier-free.

TABLE 3 (*continued*)

C. Irradiated Units[e] (*continued*)

Catalog item no.	Radioisotopes in units		Also see unit no.	Half-life[f]
	Alphabetical list	Others present		
48	Molybdenum 99	(parent of ^{99}Tc)	77	67 hr.
		^{99}Tc		10^6 y.
	Neodymium 147		35	
	Neodymium 149		35	
51	Nickel 59			15 y.
	Osmium 185		53	
	Osmium 191		53	
53	Osmium 193			17 d.
		^{185}Os		80 d.
		^{191}Os		32 hr.
55	Palladium 103		72B	17 d.
		^{111}Ag		7.5 d.
		^{103}Rh		5.6 mo.
56A	Phosphorus 32		17, 56B, 75	14.3 d.
		^{35}S		87.1 d.
56B	Phosphorus 32		17, 56A, 75	14.3 d.
		^{42}K		12.4 hr.
	Phosphorus 32	(Also available in separated form)		
	Platinum 197		30B	
	Platinum 199		30B	
	Polonium 210		9A, 9B	

[e] An irradiated unit is a specified quantity of target material that has been sealed in an aluminum case and irradiated in the pile. The unit is shipped without chemical processing. Several isotopes are present in more than one unit; reference to all units in which an isotope is found in significant quantities is given opposite the name of the isotope. Requests for smaller quantities per unit may be approved but no reduction in price will be made since production costs remain the same. In those few cases

TABLE 3 (*continued*)

C. Irradiated Units[e] (*continued*)

Radiation (m.e.v.)		Target material		Estimated quantity (mc.)	Mc./gm. element (est.)[f]	Price per unit
Beta	Gamma	Compound	Grams			
1.3	0.770, 0.815, 0.840	MoO_3	6	40	5	$12.00
0.3	None			Very small	CF	
K, e^-		Ni	10	1	0.1	33.00
0.35	Present	Os	0.55	40	73	42.00
K	None			?		
1.5	1.17			18	33	
K	None	Pd	1	Undetermined		33.00
0.8	None			10	CF	
IT	0.40					
1.69	None	S	2000	1500	CF	323.00
0.17	None			80	0.04	
1.69	None	KH_2PO_4	31	350	50	33.00
$\begin{cases} 3.58\ (75\%) \\ 2.07\ (25\%) \end{cases}$	1.51 (25%)			140	15.7	

where the value of the target material is appreciable, units are available in two sizes, the standard size and a 1/10 size at a reduced price. Irradiation units are offered with the understanding that because of variations in production setup, activities may differ 50% from the quantities estimated. See revised price list (No. 2) for additional data and additional isotopes.

[f] d. =days, hr. =hours, mo. =months, y. =years, CF =carrier-free.

TABLE 3 (*continued*)

C. Irradiated Units[e] (*continued*)

Catalog item no.	Radioisotopes in units Alphabetical list	Others present	Also see unit no.	Half-life[f]
59	Potassium 42		11, 17, 56B	12.4 hr.
60	Praseodymium 142			19.3 hr.
	Praseodymium 143		15	
64	Rhenium 186	^{188}Re		90 hr. 18 hr.
	Rhenium 188		64	
65A	Rhodium 105		65B, 67A	36 hr.
		^{97}Tc		90 d.
		^{97}Ru (parent of ^{97}Tc)		2.8 d.
		^{103}Ru		42 d.
65B	Rhodium 105		1/10 of Unit 65A	
66A	Rubidium 86		66B	19.5 d.
66B	Rubidium 86		1/10 of Unit 66A	
67A	Ruthenium 97		65A, 65B, 67B	2.8 d.
		^{97}Tc		93 d.
		^{103}Ru		42 d.
		^{105}Rh		36 hr.
67B	Ruthenium 97		1/10 of Unit 67A	
	Ruthenium 103		65A, 65B, 67A, 67B	

[e] An irradiated unit is a specified quantity of target material that has been sealed in an aluminum case and irradiated in the pile. The unit is shipped without chemical processing. Several isotopes are present in more than one unit; reference to all units in which an isotope is found in significant quantities is given opposite the name of the isotope. Requests for smaller quantities per unit may be approved but no reduction in price will be made since production costs remain the same. In those few cases

TABLE 3 (*continued*)

C. Irradiated Units[e] (*continued*)

Radiation (m.e.v.)		Target material		Estimated quantity (mc.)	Mc./gm. element (est.)[f]	Price per unit
Beta	Gamma	Compound	Grams			
{ 3.58 (75%)		K_2CO_3	22	130	10	$12.00
{ 2.07 (25%)	1.51 (25%)					
2.14	1.9	Pr_2O_3	0.085	40	550	13.00
1.0	None	Re	0.05	75	1500	12.00
2.5	0.16–1.45 complex			120	2400	
0.5	0.3	RuO_2	5	10	CF	54.00
K, e^-	0.97			0.3	CF	
K, e^-	0.22, 0.18			8	2	
{ 0.2 (95%)	0.56			3	1	
{ 0.8 (5%)						
						13.00
1.60	None	Rb_2CO_3	6.5	100	20	62.00
						34.00
K, e^-	0.22,0.18	RuO_2	5.0	10	3	68.00
K, e^-	0.97			1	CF	
{ 0.2 (95%)	0.56			10	3	
{ 0.8 (5%)						
0.5	0.3			10	CF	
						34.00

where the value of the target material is appreciable, units are available in two sizes, the standard size and a 1/10 size, at a reduced price. Irradiation units are offered with the understanding that because of variations in production setup, activities may differ 50% from the quantities estimated. See revised price list (No. 2) for additional data and additional isotopes.

[f] d. =days, hr. =hours, mo. =months, y =years, CF =carrier-free.

TABLE 3 (*continued*)

C. Irradiated Units[e] (*continued*)

Catalog item no.	Radioisotopes in unit Alphabetical list	Others present	Also see unit no.	Half-life[f]
68[h]	Samarium 153		24B	47 hr.
		^{155}Eu		2 y.
		^{155}Sm		25 mo.
		(parent of ^{155}Eu)		
69A	Scandium 46		13, 84	85 d.
		^{45}Ca		180 d.
70	Selenium 75			125 d.
72A	Silver 110			225 d.
72B	Silver 111		55	7.5 d.
		^{103}Pd		17 d.
73	Sodium 24			14.8 hr.
74	Strontium 89			55 d.
75	Sulfur 35		17, 56A	87.1 d.
76	Tantalum 182			117 d.
	Technitium 97		65A, 65B, 67A, 67B	
77	Technitium 99		48	10^6 y.
		^{99}Mo		67 hr.

e An irradiated unit is a specified quantity of target material that has been sealed in an aluminum case and irradiated in the pile. The unit is shipped without chemical processing. Several isotopes are present in more than one unit; reference to all units in which an isotope is found in significant quantities is given opposite the name of the isotope. Requests for smaller quantities per unit may be approved but no reduction in price will be made since production costs remain the same. In those few cases where the value of the target material is appreciable, units are available in two sizes, the standard

TABLE 3 (*continued*)

C. Irradiated Units[e] (*continued*)

Radiation (m.e.v.)		Target material		Estimated quantity (mc.)	Mc./gm. element (est.)[f]	Price per unit
Beta	Gamma	Compound	Grams			
0.73	0.1, 0.57 (weak)	Sm_2O_3	0.01	16	2000	$9.00
0.3	0.084			0.01	CF	
1.9	~0.3					
0.36	1.12, 0.90	Sc_2O_3	0.03	15	750	33.00
0.250	None			0.001	CF	
K, e^-	<0.3 complex, 0.5	Se	20	65	3.3	33.00
0.59	0.66, 0.90, 1.40	$AgNO_3$	7.1	35	8	33.00
1.0	None	Pd	1	10	CF	15.00
K	None			?	?	
1.4	1.4, 2.8	Na_2CO_3	0.3	20	150	12.00
1.5	None	$Sr(NO_3)_2$	33	1.5	0.11	33.00
0.17	None	Available in separated form (see S–4)				
0.53	1.13, 1.22 <1.0 complex	TaO_2	0.22	40	215	33.00
0.3	None	MoO_3	6	0.00001	CF	33.00
1.3	0.770, 0.815, 0.840			45	11	

size and a 1/10 size at a reduced price. Irradiation units are offered with the understanding that because of variations in production setup, activities may differ 50% from the quantities estimated. See revised price list (No. 2) for additional data and additional isotopes.

[f] d. =days, hr. =hours, mo. =months, y. =years, CF =carrier-free.

[h] Target material to be furnished by requester.

TABLE 3 (*concluded*)

C. Irradiated Units[e] (*continued*)

Catalog item no.	Radioisotopes in unit Alphabetical list	Others present	Also see unit no.	Half-life[f]
	Tellurium 127		37	
	Tellurium 129		37	
	Tellurium 131		37	
80	Thallium 206			2.7 y.
	Tin 113		3C	
	Tin 121		3C	
	Tin 123		3C	
84	Titanium 51	^{46,47,48}Sc probable		72 d.
85A	Tungsten 185	^{187}W	85B	77 d. 24 hr.
85B	Tungsten 187	^{185}W	85A	24 hr. 77 d.
90	Yttrium 90			62 hr.
91	Zinc 65	^{69}Zn		250 d. 1 hr., 13.8 hr.
	Zinc 69		91	
92	Zirconium 95			65 d.

[e] An irradiated unit is a specified quantity of target material that has been sealed in an aluminum case and irradiated in the pile. The unit is shipped without chemical processing. Several isotopes are present in more than one unit; reference to all units in which an isotope is found in significant quantities is given opposite the name of the isotope. Requests for smaller quantities per unit may be approved but no reduction in price will be made since production costs remain the same. In those few cases where the value of the target material is appreciable, units are available in two sizes, the standard

TABLE 3 (concluded)

C. Irradiated Units[e] (continued)

Radiation (m.e.v.)		Target material		Estimated quantity (mc.)	Mc./gm. element (est.)[f]	Price per unit
Beta	Gamma	Compound	Grams			
0.58	None	$Tl(NO_3)_3$	10	7	1.2	33.00
0.36	1.0	TiO_2	19	1	0.9	33.00
0.7	None	WO_3	1.8	10	7	33.00
0.6,1.3	0.086–0.94 complex			500	350	
0.6,1.3	0.086–0.94 complex	WO_3	0.12	40	420	12.00
0.7	None			0.2	21	
2.16	None	Y_2O_3	1.1	100	115	33.00
$0.4\ \beta^+$ (1%) K, e^- (99%)	1.14	Zn	8	15	1.9	33.00
IT^i 1.0	0.439			60	7.5	
1.0, 0. 394	0.73, 0.92	$Zr(OH)_4$	22	12	9	12.00

size and a 1/10 size at a reduced price. Irradiation units are offered with the understanding that because of variations in production setup, activities may differ 50% from the quantities estimated. See revised price list (No. 2) for additional data and additional isotopes.

[f] d. =days, hr. =hours, mo. =months, y. =years, CF =carrier-free.

[i] IT =isomeric transfer.

TABLE 4

Cyclotron Yields of Artificial Radioelements[4]

Isotope	Half-life	Reaction	Yield, μc./μa./hr.		
			8 m.e.v. d	14 m.e.v. d	22 m.e.v. d
$^{3}_{1}H$	31 y.	Be $(d, {}^{3}H)$	——	0.1	——
$^{11}_{6}C$	21 min.	B (d,n)	500	——	——
$^{14}_{6}C$	5,100 y.	N (n,p)	——	0.0005	——
$^{13}_{7}N$	9.93 min.	C (d,n)	1000	——	——
$^{22}_{11}Na$	3 y.	Mg (d,a)	——	1.0	——
$^{24}_{11}Na$	14.8 hr.	Na (d,p)	——	——	1500
$^{27}_{12}Mg$	10.2 min.	Mg (d,p)	500	2800	——
$^{32}_{15}P$	14.3 d.	P (d,p)	30	120	——
$^{35}_{16}S$	88 d.	Cl (d,a)	——	——	8.4
$^{38}_{17}Cl$	37.0 min.	Cl (d,p)	1000	——	——
$^{42}_{19}K$	12.4 hr.	K (d,p)	——	50.0	107
$^{42}_{19}K$	12.4 hr.	A (a,pn)	——	——	——
$^{42}_{19}K$	12.4 hr.	Ca (d,a)	——	——	3.3
$^{45}_{20}Ca$	180 d.	Ca (d,p)	——	0.01	——
$^{54}_{25}Mn$	310 d.	Fe (d,a)	——	(1.0)	——
$^{59}_{26}Fe$	47 d.	Fe (d,p)	——	0.03	——
$^{55}_{26}Fe$	4 yr.	Mn $(d,2n)$	——	——	(0.02)
$^{57}_{27}Co$	270 d.	Fe (d,n)	——	(1.0)	——
$^{64}_{29}Cu$	12.8 hr.	Cu (d,p)	——	3000	——
$^{65}_{30}Zn$	250 d.	Cu $(d,2n)$	——	(0.5)	——
$^{74}_{33}As$	16 d.	Ge (d,n)	——	2	——
$^{86}_{37}Rb$	19.5 d.	Rb (d,p)	——	4.3	——
$^{86}_{37}Rb$	19.5 d.	Sn (d,a)	——	1.0	——
$^{85}_{38}Sr$	66.5 d.	Rb $(d,2n)$	——	(0.13)	(0.60)
$^{89}_{38}Sr$	55 d.	Sr (d,p)	——	10.4	——
$^{86}_{39}Y$	108 d.	Sr $(d,2n)$	——	(0.10)	(1.0)
$^{89}_{40}Zr$	81 hr.	Y $(d,2n)$	——	7.0	75.0
$^{95}_{40}Zr$	63 d.	Zr (d,p)	——	0.15	——

TABLE 4 (*continued*)

Cyclotron Yields of Artificial Radioelements[4]

| Isotope | Half-life | Reaction | Yield, μc./μa. hr. | | |
			8 m.e.v. d	14 m.e.v. d	22 m.e.v. d
$^{90}_{41}$Cb	18 hr.	Mo (d,α)	——	*2.4*	——
$^{95}_{41}$Cb	36 d.	Mo (d,α)	——	*0.05*	——
$^{91}_{41}$Cb	60 d.	Zr (d,n)	——	*(1.0)*	——
$^{121}_{52}$Te	125 d.	Sb $(d,2n)$	——	*(0.16)*	——
$^{131}_{53}$I	8 d.	Te (d,n)	*1.0*	*20.0*	——
$^{133}_{56}$Ba	39.6 hr.	Cs $(d,2n)$	——	*(100.0)*	——
$^{210}_{84}$Po	136 d.	Bi (d,n)	——	*2.0*	——
$^{210}_{84}$Po	136 d.	Pb $(\alpha,2n)$	——	——	——
$^{211}_{85}$At	7.5 hr.	Bi $(\alpha,2n)$	——	——	——
$^{233}_{91}$Pa	27.4 d.	Th $(d,2n)$	——	——	*(20.8)*

The cyclotron is, however, a much more versatile instrument in producing isotopes than the pile because of the great variety and energy of the nuclear projectiles which it can employ. The cyclotron is complementary to the pile, and is absolutely essential to many types of investigations. Certain useful isotopes, such as ^{7}Be, ^{22}Na, ^{49}V, ^{54}Mn, and ^{74}As, are not produced in significant amounts with a pile, but are readily prepared with a cyclotron. Beside deuterons, neutrons, alpha particles, and protons are used as bombarding agencies in the cyclotron. The yield obtained in the preparation of a great number of isotopes by the cyclotron is given in Table 4 (compiled by Hamilton) in which the atomic number, mass number, half-life, and the type of reaction are stated. The yields are given in terms of microcuries per microampere hour. A microcurie is the absolute number of disintegrations per second of the artificially prepared radioelement, or 3.7×10^4 disintegrations per second. This definition, however, does not apply to the radioelements whose yield values are enclosed in parenthesis, since they are all substances decaying by K-capture and the measured radiation contains varying proportions of x-rays, γ-rays, and internally converted electrons. In such instances, the value of the microcurie is a compa-

[4] J. G. Hamilton, personal communication.

rable one and simply indicates that the amount of ionization produced is equivalent with the measuring device employed to that from one microcurie of the radioactive standard. A Lauritsen electroscope with a thin-walled aluminum window was employed for these measurements. The total air equivalent of the window together with the distance of the sample from the instrument totalled 4 cm. The instrument was calibrated by means of UX_1 standards which were covered with a sufficient thickness of aluminum foil to screen out most of the soft UX_1 beta rays and approximately 15% of the more energetic UX_2 beta particles.

The values presented in the table include yields for deuterons at energies of 8, 14, and 22 m.e.v. In the case of ^{14}C, the value given is a calculated one assuming a bombardment of 100 gallons of saturated ammonium nitrate. The italicized yield values are believed to be accurate to ±25%. The nonitalicized yield values are subject to much greater error. When applying alpha particles of 44 m.e.v. yields as high as 80 in the case of ^{42}K and 1000 in the case of ^{211}At (microcuries per microampere per hour) were observed.

A reduction of the energy of deuterons to 5 m.e.v. leads to a ^{32}P yield of 15 microcuries per microampere per hour. Only a small fraction of the deuteron beam in a cyclotron is utilized in nuclear processes. Even at bombarding energies as high as 16 m.e.v. no more than 0.2% of the incident deuterons are captured by a target of low atomic number such as beryllium.

While most of the radioactive isotopes are prepared by deuteron bombardment in the cyclotron, in some cases, as in that of the formation of ^7Be according to the equation:

$$^7_3Li + {}^1_1H \rightarrow {}^7_4Be + {}^1_0n$$
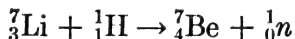
protons of 10 m.e.v. or more are advantageously used. Bombardment with 40 m.e.v. α-particles is bound to be very effective[4] when producing ^{42}K or ^{82}Br. The corresponding equations are:

$$^{40}_{18}A + {}^4_2He \rightarrow {}^{42}_{19}K + {}^1_1H + {}^1_0n$$
$$^{80}_{34}Se + {}^4_2He \rightarrow {}^{82}_{35}Br + {}^1_1H + {}^1_0n$$
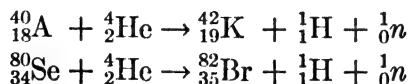
High-voltage plants can also be used in the production of radioactive tracers. Mainly deuterons are accelerated by the high voltage, which by impact on a light element, as lithium, beryllium, or boron, produce neutrons.

CHAPTER III

Determination of Radioactivity

I. Measurement of Activity of Radiophosphorus

In the application of radioactive indicators in biology we are often faced with the problems of determining the rate of excretion and distribution of labeled atoms in the organism, of determining the organic compounds in which the labeled atoms are to be found, and of determining the rate at which they are incorporated and released. The problem often put to the analyst is one of comparing the radioactivity of a known aliquot of the tagged substance administered with that of a tissue sample or with a fraction extracted from the tissue.

Many radioactive substances decay to an appreciable extent during the experiment. The rates of decay of ^{32}P, ^{24}Na, and ^{42}K are shown in Tables 14 to 16, pages 80 and 81. We can usually avoid correcting for decay rate by comparing the activity of the sample to be investigated, for example, the activity of a ^{32}P-containing sample, with that of a standard preparation containing the isotope. As a standard preparation an aliquot of the preparation administered is often taken. By this procedure the disturbing effect of decay is eliminated. Another source of error is the absorption of the β-rays emitted by the radioactive isotope in the sample, the absorption being dependent on the thickness, viz., in first approximation, on the weight per cm.^{2} of the sample. (Half the β-radiation emitted by ^{32}P is absorbed by an 0.4-mm. aluminum layer. Its absorption coefficient in aluminum is 22.9. The range[1] of the β-rays in aluminum is 2.9 mm.[1]) This error can best be eliminated by comparing samples of identical chemical composition and of equal weight.

The electrons emitted in the β-disintegrations of ^{32}P are partially retarded in the immediate vicinity of the atomic nucleus — thus giving rise to x-rays, the so-called internal "Bremsstrahlung" — and lose further velocity in the surrounding atoms, which likewise leads to x-radiation,

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[1] J. Govaerts, Mém. soc. roy. sci. Liége, **5**, No. 2 (1941). W. F. Libby, Anal. Chem., **19**, 2 (1947).

the *external Bremsstrahlung*. While ^{32}P nuclei do not emit γ-rays, the presence of *Bremsstrahlung* can readily be observed when strong ^{32}P samples are employed. The *Bremsstrahlung*, released by β-rays from ^{32}P, amounts to 1 γ-quantum per 25 β-particles when the retardation takes place in lead. When absorption takes place in paraffin and aluminum the intensities of the *Bremsstrahlung* are 1/12 and 1/6, respectively, of the intensity for retardation in lead.[2a] Due to the emission of *Bremsstrahlung*, special precautions must be taken when strong samples of ^{32}P are mailed.

In the investigation of phosphorus metabolism, the sample containing ^{32}P is often dissolved by wet ashing, inactive sodium phosphate is added, and the phosphorus is precipitated as magnesium ammonium phosphate. Sulfuric and nitric or sulfuric and perchloric acids are most often used as ashing agents; occasionally sulfuric acid and hydrogen peroxide are employed. By adding a corresponding amount of inactive sodium phosphate to the solution containing the ashed samples we can obtain magnesium ammonium phosphate precipitates of equal weight. Samples of about 70 mg. were found to be convenient; such samples can easily be uniformly distributed on an aluminum dish 12 mm. in diameter and 2 mm. deep.[2] Dishes are punched out of a 100-μ aluminum foil and fit accurately into the hole of the slide of the counting arrangement (see page 68). If the weight of the samples, while having the same ^{32}P content, should differ by a few milligrams, the accuracy of comparison of the samples would not be greatly affected, as is seen in Table 5.

TABLE 5

Effect of Addition of Sodium Phosphate on Activity of Labeled Phosphate[2]

Sodium phosphate, mg.[a]	Measured activity	Sodium phosphate, mg.[a]	Measured activity
50	1	200	0.87
100	0.97	250	0.79
150	0.92	300	0.72

[a] All samples have the same ^{32}P content.

When the activity of magnesium ammonium phosphate preparations is to be compared, the weight of the lighter sample can be increased to

[2] H. Levi, *Acta Physiol. Scand.*, **2**, 311 (1941).

[2a] E. McMillan, *Phys. Rev.*, **47**, 801 (1935). See also K. Siegbahn, *Arkiv Mat. Astron. Fysik*, **A34**, No. 6 (1947).

the weight of the heavier one before precipitating the magnesium compound by adding to the solution containing radiophosphate a corresponding amount of inactive sodium phosphate. In cases in which comparison of samples of equal weight is not feasible, the values obtained are corrected according to the figures given in the above table. An increase in the weight of the sample to be measured leads to an increased absorption of the rays emitted by the sample itself, but simultaneously a greater percentage of the rays emitted by the sample, which now get nearer the window of the counter (see page 70), is registered by the counter. For this reason, the error due to increased absorption of the radiation by an increase in weight of the sample is partly compensated.

The magnesium ammonium phosphate sample precipitated in a beaker can be filtered and, after being dried at 40° C., transferred to an aluminum dish. A more time-saving procedure is filtration of the solution containing the precipitate through a porcelain crucible with a perforated bottom and transfer of the dried precipitate to the aluminum dish. Finally, transfer can be entirely avoided by filtering the precipitate through a perforated aluminum dish (see page 74) covered by a thin sheet of filter paper. The precipitate, usually having an uneven surface, is, after being washed with dilute ammonia, straightened out by use of a glass rod. The latter procedure is to be recommended.

Phosphate can also be precipitated as molybdenum salt. Although radiophosphorus is representative of radioactive indicators emitting fairly hard β-rays, in view of the high absorbing power of molybdenum for the β-rays emitted by the sample, this procedure is seldom used. Several other elements can easily be isolated quantitatively from digested tissue samples. The halogens, for example, can be obtained as silver compounds. In their investigations with radioiron, Hahn and his associates[3] precipitated the iron electrolytically prior to the activity measurement. The formation of a copper complex of diphenylthiocarbazone (dithizone) provides the basis for the isolation of radioactive copper prior to the activity measurement.[4]

We often lack a simple procedure for concentrating the radioactive isotope prior to the activity measurement. In such cases, the activity of a solution of the sample or of substances extracted from the sample is measured. The activity of radiosodium- or radiopotassium-containing samples is often determined by this method; but occasionally the activity

[3] P. F. Hahn, W. F. Bale, and W. M. Balfour, *Am. J. Physiol.*, **135**, 600 (1942).
[4] M. O. Schultze and S. J. Simmons, *J. Biol. Chem.*, **142**, 97 (1942).

of even other radioactive substances, as, for example, that of radio-phosphorus, is measured in solution.[5] After wet ashing, the activity of the solution obtained is measured and, thus, the work involved in precipitating the phosphate and placing the dried precipitate in the aluminum dish is avoided. However, this procedure can be used only if the sample is fairly active, since an appreciable part of the radiation is absorbed by the solution. Furthermore, in the investigation of phosphorus metabolism small fractions of phosphatides, nucleic acids, or other compounds must often be isolated; the activity of these is more conveniently measured in solid state, usually after transformation to magnesium ammonium phosphate. The activity of small amounts of nucleic acid (down to 0.5 mg.), for example, can be compared with sufficient accuracy without converting the phosphorus to magnesium ammonium phosphate.[6]

When the radiosodium or radiopotassium content of biological samples is not determined by extracting the alkali of the sample with nitric acid and by measuring the activity of the solution obtained, the activity of the dried sample is compared with a standard sample of equal weight and, as far as possible, of equal composition. This method is also used in the determination of other radioactive substances. Potassium can also be precipitated as potassium cobaltic hexanitrite or perchlorate; zinc as carbonate.[7]

When determining the activity of red corpuscles, the radioisotope can be isolated from the corpuscles and determined, or the activity of the dried sample can be compared with the activity of a standard sample. For the standard sample, another red corpuscle sample is preferably used, and the weight of the sample to be measured is brought up to the weight of the standard sample (or vice versa) by addition of inactive red corpuscles. It is advisable to pulverize the red corpuscles, and also plasma samples, prior to the activity measurement. Comparison of the results of the activity measurements of dried and pulverized samples with that of the magnesium ammonium phosphate fractions isolated from the corpuscles gave satisfactory results.[8]

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[5] W. F. Bale, F. L. Haven, and M. L. Le Fevre, *Rev. Sci. Instruments*, **10**, 193 (1939).

[6] J. Ottesen, *Acta Physiol. Scand.*, **10**, 214 (1945).

[7] G. E. Sheline, I. L. Chaikoff, H. B. Jones, and M. L. Montgomery, *J. Biol. Chem.*, **147**, 409 (1943).

[8] G. Nylin, *Arkiv Kemi Mineral. Geol.*, **A20**, 17 (1945).

II. Measurement of Activity of Radiosulfur

Radiosulfur is representative of radioactive isotopes emitting soft rays. The β-radiation emitted by ^{35}S is much softer than that emitted by ^{32}P and some frequently used tracers, as seen in Table 6.

TABLE 6

Absorption Data of Some Tracers

Isotope	Half-life	Maximum energy of β-rays, K.e.v.	Al range, mg./cm.^{2}	Half-thickness, mg./cm.^{2}
T	31 y.	15	0.23	0.03
^{11}C	20.5 min.	950	390	54
^{14}C	5100 y.	154	20	2.8
^{18}F	112 min.	700	260	36
^{22}Na	3.0 y.	580	215	30
^{24}Na	14.8 hr.	1400	620	86
^{31}Si	170 min.	1800	860	120
^{32}P	14.3 d.	1690	800	110
^{35}S	87 d.	167	13.5	1.9

Sulfur is isolated prior to measurement of its radioactivity as barium sulfate or as benzidine sulfate. Advantage of the benzidine method is that these precipitates are easier to collect than are the barium precipitates. The particle size is more uniform and the procedure can be carried out more quickly and efficiently. A disadvantage of the method lies in the weight of the benzidine precipitate, which is 1.56 times that of the barium. The dilution effect of nonradioactive sulfur added on the activity of the sample measured by a modified Lauritsen electroscope is seen in Table 7.

TABLE 7

Effect of Addition of Inactive Sulfur on Activity of Radiosulfur[9]

Dilution of 10-mg. sample by inactive sulfur	Cystine equivalent, mg.	Ratio of radioactivity to background
1	0.80	250
25	0.032	10
100	0.008	3
1000	0.0008	0.2

[9] H. Tarver and C. L. A. Schmidt, *J. Biol. Chem.*, **130**, 67 (1939).

Henriques et al.,[10] state that both the bell-type mica window counter and the Lauritsen electroscope will readily detect 10^{-4} microcuries ^{35}S. Since the β-particles emitted by ^{35}S have a maximum energy of 167,000 e.v. only, a considerable fraction of these particles ·is absorbed in the sulfate precipitate. The extent of absorption in a benzidine sulfate precipitate is seen in Table 8, in which the weight of the precipitate and the reciprocal weight correction factor are stated, taking as a basis a precipitate having a weight of 3.25 mg. per cm.^{2} Net measured activity is to be divided by these factors.

TABLE 8

Self-Absorption of ^{35}S Beta Particles in Benzidine Sulfate[10]

Ppt. wt., mg./cm.²	G-M counter reciprocal wt. correction factor	Ppt. wt., mg./cm.²	G-M counter reciprocal wt. correction factor	Ppt. wt., mg./cm.²	G-M counter reciprocal wt. correction factor
0.46	1.370	4.65	0.865	8.80	0.575
0.93	1.290	5.10	0.825	9.25	0.555
1.40	1.205	5.55	0.790	9.75	0.535
1.85	1.150	6.00	0.754	10.20	0.520
2.30	1.095	6.50	0.715	10.70	0.500
2.80	1.050	6.95	0.680	11.10	0.480
3.25	1.000	7.40	0.650	11.60	0.465
3.70	0.955	7.90	0.625		
4.15	0.910	8.35	0.600		

III. Measurement of Activity of ^{14}C

In view of the great importance of ^{14}C in metabolic studies, extensive efforts were made to work out suitable methods for measuring the intensity of the soft radiation emitted by this isotope. The maximum energy of β-rays emitted by ^{14}C amounts to 0.154 m.e.v. only. It is convenient to precipitate the radiocarbon as barium carbonate, such a precipitate being easier to handle than calcium carbonate, which would be preferred because of its higher content; still more favorable would be samples of lithium carbonate or of another light carbonate compound. When measuring the activity of $^{14}BaCO_3$ samples an increase in the weight of the sample above 10 mg. per cm.^{2} increases the number of counts to a minor extent only and an increase above 25 mg. per cm.^{2}

[10] F. C. Henriques, Jr., and C. Margnetti, Ind. Eng. Chem., Anal. Ed., **18**, 415 (1946).

is entirely futile because the β-rays emitted by the deeper layers are entirely absorbed in the sample, as indicated by Table 9.

TABLE 9

Absorption by the Sample of Beta Rays Emitted by ^{14}BaCO$_3$ and Effect of Increasing Sample Weight on Number of Counts[11]

BaCO$_3$, mg./cm.^{2}	$J(x)$[a]	Measured activity	BaCO$_3$, mg./cm.^{2}	$J(x)$[a]	Measured activity
0	1.000	0	11	0.304	947
1	0.866	245	15	0.230	978
2	0.761	431	16	0.217	983
3	0.660	560	20	0.176	995
4	0.586	663	21	0.168	996
5	0.527	745	24	0.147	999
10	0.330	934	25	0.141	1000

[a] The function $J(x)$ represents the variation with sample layer thickness of the apparent specific activity.

The data in Table 9 were obtained by Yankwich *et al.*, with a counter[12] which had a mica window weighing 3.6 mg. per cm.^{2} If a barium carbonate sample having a thickness of 2.00 mg. per cm.^{2} has an activity of 1000 counts per minute, the activity which would be observed if the thickness of the sample material were infinitesimal is $1000/0.761 = 1314$ counts per minute. If the geometry of the counter is such that 20% of the particles emitted reaches the effective part of the tube, the total number of β-particles emitted by the sample proves to be 6570.

Hendricks[13] and co-workers developed a method for mounting samples of Ba^{35}SO$_4$ which in practice can be relied upon to within an error of about 4%. Their method has been extended to Ba^{14}CO$_3$.

A tared aluminum disc, 0.15 mm. thick and 4.5 cm. in diameter, weighing about 700 mg., is placed in the bottom of a machined brass cup. A thick concentric brass sleeve is placed on top of the disc and their common edge sealed by screwing on a pressure ring. A circular area of 11.52 cm.^{2} is left exposed at the bottom of the cup thus formed. The precipitate, or other sample material, is ground to a fine powder in

[11] P. E. Yankwich, T. H. Norris, and J. Huston, *Anal. Chem.*, **19**, 439 (1947).

[12] P. E. Yankwich, G. K. Rollefson, and T. H. Norris, *J. Chem. Phys.*, **14**, 131 (1946).

[13] R. H. Hendricks, L C. Bryner, M. V. Thomas, and J. O. Ivie, *J. Phys. Chem.*, **47, 469** (1943).

an agate mortar under 95% ethanol. When the grinding is complete (2 to 5 minutes), the slurry is washed into the cup. The powder is allowed to settle and the alcohol evaporated by placing the plate holder on a hot plate.

With $BaSO_4$ the above directions result in the preparation of plates which are uniform to the eye and which can be made to approach about 4% error in reproducibility. When $BaCO_3$ is used the results are not as good. The particles are somewhat less dense than $BaSO_4$ and there is a much greater tendency for them to creep with the meniscus of the alcohol as it evaporates. When the sample weighs less than 20 mg. or about 2 mg. per cm.^{2}, this results in a plate which is spotty and unevenly coated. The effect with samples of this weight is not so pronounced as it would be with heavier samples because with small samples a relatively small fraction of the radiation is cut out by the sample itself; the result is a plate with a high edge, uneven, and impossible to reproduce.

In the first refinement of the method, the plate holder, containing the ground sample, was heated on a hot plate until the alcohol began to boil. The holder was then placed under an infrared heat lamp so adjusted that the alcohol was just below its boiling point. When most of the alcohol had evaporated, the slurry, now technically a "slip," was still mobile. The plate holder was then removed from under the heat lamp and agitated gently until the sample layer became too stiff to move. The agitation pattern was that of a many-leaved rose, accomplished by "rattling" the plate holder between the thumb and middle finger.

It was found that the high edges could be leveled if the plate holder was tapped sharply against a hard surface while the "slip" was still mobile.[14]

Barium carbonate ($^{14}BaCO_3$) should not be left standing in a moist atmosphere because an interchange takes place between some of the sample carbonate and carbon dioxide of the atmosphere.[15]

When large amounts of radiocarbon of small specific activity are to be measured, it may be more advantageous to convert the carbon into carbon dioxide and to measure its activity with the ionization chamber. The method worked out by Henriques et al.,[10] to measure activity of

[14] P. E. Yankwich, personal communication. P. E. Yankwich, G. K. Rollefson, and T. H. Norris, *J. Chem. Phys.*, **14**, 131 (1946).

[15] W. Armstrong and Y. Schubert, personal communication.

carbon is based on the insertion of carbon dioxide gas into a quartz ionization chamber attached to a Lauritsen electroscope. It is capable of detecting the presence of 3×10^{-5} microcurie of ^{14}C in 20 millimoles of carbon dioxide, which corresponds to 4 grams of barium carbonate. Twenty millimoles of carbon are normally present in 2 to 4 grams of animal tissue. The labeled carbon dioxide obtained by burning the tissue is transferred to a mixing bulb and the amount of nonradioactive carbon dioxide gas required to bring the total amount to 20 millimoles is added. The gas is then introduced into the evacuated ionization chamber. By using an ionization chamber 100 milliliters in volume and containing 50 milligrams of carbon as carbon dioxide at atmospheric pressure, Janney and Moyer[15a] find that the measurement of a sample producing 75 counts per minute per milligram carbon in the counter mentioned above is measured during a given time more accurately with the ionization chamber than with the counter.

Another method which eliminated the disturbing effects of the absorption of the β-rays by the sample is based on the introduction of carbon dioxide into the Geiger-Müller tube by Miller[16] and by Libby.[1]

Miller used a conventionally designed glass envelope of the gamma counter type with a 6-mil tungsten center wire and a cathode of silver chemically deposited on the inner wall and then coated with colloidal graphite. They are tabulated near one end with a stopcock and ground joint for filling. Counters of a wide range of sizes are found to work well. The sizes of some of the counters used are described in Table 10.

TABLE 10
Description of the Counter Tubes[16]

Counter No.	Length, cm.	Inside diameter, cm.	Total volume, ml.	Cathode length, cm.	Cathode volume, ml.	Total volume cathode volume
18	33	3.8	316	28	301	1.050
19	16	1.2	18.2	13.0	13.5	1.340
20	16	2.1	60.5	14.1	48.8	1.240
22	21	2.1	74.2	18.4	63.7	1.165
23	31	2.1	113.3	28.6	99.2	1.142

The filling gas is the sample of active carbon dioxide at any pressure from 10 to 50 cm. mercury with, in all cases, sufficient carbon disulfide vapor to represent 2 cm. pressure within the counter tube. Threshold voltages range from 1800 to 4500, depending on counter diameter and carbon dioxide pressure. The plateau

[15a] C. D. Janney and B. J. Moyer, personal communication.

[16] W. W. Miller, *Science*, **105**, 123 (1947).

starts at 80–90 v. above threshold, extends for 200 v., and has a slope of the order
of 2% per 100 v. The optimum operating range is about 160 v. above threshold.
These counter tubes have been used in conjunction with a stabilized high-voltage
power supply having an output up to 5000 v., modified Neher-Pickering preampli-
fier, scale of 128, Cenco register, and electric timer.

Under these conditions it is found that within these pressure limits the measured
counting rate per unit amount of active carbon dioxide is completely independent of
the total pressure of carbon dioxide and is thus a function only of the amount of
activity within the counter tube. It is further found that, if the counting rate is
corrected by the ratio of the total volume of the counter to the volume defined by
the cylindrical cathode, all counter tubes within the above size range show the same
response to equal amounts of activity within the counters. The nonlinearity cor-
rection for these counters is relatively large, amounting to 12% loss at 10,000 c.p.m.
This correction is made from a calibration curve prepared by adding increment
amounts of active CO_2 to a counter tube and plotting observed counter rate less
background against arbitrary units of active carbon dioxide in the counter tube.

TABLE 11
Activity Measurements[16]

Sample No.	Radio-CO_2 (micromoles)	CO_2 (millimoles)	Counter No.	CO_2 pressure in counter, cm. Hg.	Threshold voltage	Observed counting rate, c.p.m.	Background rate, c.p.m.	Linearity correction factor	Final corrected counting rate	c.p.m./μm. radio-CO_2
1	42.2	5.67	18	33.8	4060	2090	320	1.000	1860	44.0
2	36.2	0.159	19	20.1	2920	1420	40	1.000	1850	51.1
3	56.1	3.10	20	9.8	4320	2200	70	1.005	2655	47.3
4	131	1.18	23	21.6	3555	5230	159	1.065	6170	47.1
5	201	1.15	22	34.0	3720	7130	99	1.138	9320	46.3
6	254	3.20	18	20.5	3540	9840	301	1.203	12040	48.8
7	348	0.159	19	52.3	4390	9550	39	1.295	16500	47.4

The measurements presented in Table 11 were made on samples prepared by
mixing measured amounts of inert and active carbon dioxide. The samples of
activity were all drawn from the same bulb containing a preparation of radioactive
carbon dioxide. The prepared sample was distilled into a counter tube attached
to the glass system with a ground joint by immersing one end of the counter tube in
liquid nitrogen. The proper amount of inert carbon disulfide vapor to represent
2 cm. pressure in the counter was then added by means of a "doser," after which
the counter was detached from the line, allowed to warm to room temperature,
placed in a lead housing; the preamplifier leads were attached, and the counting
rate was determined at 160 v. above threshold. Background rate is determined in
the same counter filled with inert carbon dioxide.

The final corrected counting rate is obtained by subtracting background from the observed rate, multiplying this by the ratio of the total volume of the counter to the cathode volume from Table 10 to correct for that fraction of the sample that is not within the sensitive volume of the counter tube, and then correcting this figure for the nonlinearity of the counters with the factor taken from the empirically determined curve previously referred to. The data in the last column of Table 11 show that, with an average deviation of 3%, the system allows the direct comparison of activities of a large range of sample sizes. The sample size range from 0.1 to 9 millimoles of carbon is represented by 10 cm. pressure in the smallest counter to 50 cm. in the largest. In terms of $BaCO_3$, on which measurements are usually made by solid counting techniques, this represents 20 mg. to 2 grams. Sample 7, when removed from the counter, precipitated as $BaCO_3$ on a 2-cm. filter, and measured on a bell-type β-counter with a 4-mg. per cm.^{2} window, gave an observed counting rate, less background, of 50 c.p.m. This is 2.5 $\times$ background as against 250 $\times$ background on the sample when measured as a gas.

Barium carbonate containing as much as 8% ^{14}C has been prepared. One part of such carbon, even if diluted by 10^8 parts of inactive carbon, can still be measured. This figure should be compared with the 5000-fold dilution that is possible with the use of the stable isotope ^{13}C.

IV. Measurement of Activities of ^{55}Fe and ^{59}Fe in the Same Sample

It may be of interest to label red corpuscles with both radioactive iron isotopes, ^{59}Fe and ^{55}Fe.* The problem of the separate measurements of the activity of the two isotopes then occurs.[17] One of the isotopes, ^{59}Fe, has a half-life of 47 days and emits γ-rays and low-energy β-rays. The other, ^{55}Fe, has a half-life of about 5 years and emits low-energy x-rays only.

^{59}Fe (47-day half-life) disintegrates by two similar processes, each with about the same probability of occurrence. In one case a β-ray, or energetic electron, is emitted with 0.46 m.e.v. maximum energy, followed by a γ-ray of 1.10 m.e.v. In the alternate method of disintegration the β-ray has a maximum energy of 0.26 m.e.v. and is followed by a γ-ray of 1.30 m.e.v. In either case, the β-rays emitted have an average energy about one-third of maximum. The average energy of all the β-rays is then only about 0.12 m.e.v.[18]

* Double or triple labeling techniques may be of interest in metabolic studies. For instance, ^{11}C, ^{13}C, and ^{14}C can be introduced into different carbon groups of a molecule.

[17] W. C. Peacock, R. D. Evans, J. W. Irvine, Jr., W. M. Good, A. F. Kip, S. Weiss, and J. G. Gibson, *J. Clin. Investigation*, **25**, 605 (1946).

[18] M. Deutsch, J. R. Downing, L. G. Elliott, J. W. Irvine, Jr., and A. Roberts, *Phys. Rev.*, **62**, 3 (1942).

[55]Fe (5-year half-life) disintegrates into [55]Mn, a stable isotope, when the nucleus captures an electron from one of the inner orbits of the atom. This process, "orbital electron capture," leaves a vacancy in one of the inner electron shells which is subsequently filled by an electron from an outer shell. As a result, an x-ray characteristic of the [55]Mn atom may be emitted. The most abundant and most energetic x-ray emitted has an energy of 5.9 kv. ($K\alpha_1$ line of manganese). One of these x-rays leaves the atom in about 24% of the disintegrations.

Due to the hardness of x-rays emitted by ^{59}Fe, a beryllium window 0.41 mm. thick transmits 55% of the radiation. Such a window absorbs more than 98% of the soft β-radiation emitted by [59]Fe. By using a beryllium window we thus almost exclusively measure the radiation of [59]Fe. Argon gas at a pressure of 60 cm. is used in these counters since x-rays are absorbed to a much higher extent in argon than in helium; the thickness in centimeters necessary to reduce the intensity of the x-rays to half is 6000 times greater in helium (at S.T.P.) than in argon (at S.T.P.). Due to this fact, a counter with a beryllium window thick enough to stop 47-day iron β-rays and filled with argon gas at a pressure of 60 cm. mercury detects about 30 times as many x-rays as the 10-μ mica window counter filled with helium at a pressure of 70 cm. mercury.

A 10-μ mica window counter filled with helium at a pressure of 70 cm. mercury is used in the measurement of the activity of [59]Fe. This hardly registers the presence of [55]Fe. 75% of the beta radiation emitted by ^{59}Fe penetrate the mica window.[18a] The fraction of β-rays absorbed[19] within a source weighing not more than 2.6 mg. per cm.[2] is negligible; approximately 10% of the radiation is absorbed in a source weighing 5.2 mg. per cm.[2] An even, thin layer of iron can easily be obtained by electrolysis.

V. Electrolytic Separation of Radioiron

The blood or tissue sample digested with concentrated sulfuric and perchloric acids is neutralized with 40% sodium hydroxide solution. The iron is then precipitated as ferrous sulfide by alkalinizing the solution with ammonium hydroxide and saturating it with hydrogen sulfide. The precipitate is centrifuged off and decomposed with 0.5 ml. 6 N sulfuric acid, and all hydrogen sulfide is expelled by gentle boiling for one minute. The solution is made almost neutral by addition of 6 N

[18a]W. C. Peacock and W. M. Good, *Rev. Sci. Instruments,* **17,** 255 (1946).

[19] J. F. Ross and M. A. Chapin, *Rev. Sci. Instruments,* **13,** 77 (1942).

ammonium hydroxide. Ferrous ammonium oxalate is then formed by adding 20 ml. saturated ammonium oxalate solution. Electrolytic separation is carried out from this solution.

The size and shape of the copper foil used as a cathode depends on the size and shape of the β-ray counter window. Ross and Chapin[19] (see also Hahn[20]), using circular cathodes 2.5 cm. in diameter, state that the amount of iron remaining in the solution when depositing 5 mg. is not more than 0.01 to 0.02 mg. They find also that, even when increasing amounts of stable iron are added to the radioactive iron, significant absorption of the β-rays occurs after addition of more than 10 mg. stable iron.

VI. Measurement of Activity of Tritium

The β-radiation emitted by tritium (^{3}H) is even softer than the β-radiation of ^{14}C and ^{35}S. The hardest rays emitted by ^{3}H have an energy of not more than 15000 e.v. The measurement of such soft rays is much facilitated by the fact that tritium can easily be converted into water and that water vapor at a pressure of 1 to 2 mm. of mercury may be introduced into a Geiger-Müller counter tube without interfering with its performance; consequently, the activity of tritium may be measured in the form of HTO vapor.[21,22] In such measurements background determinations are carried out in an identical manner with ordinary water substituted for the active water component. Since evacuation of the counter tube is insufficient to obtain a return to normal background values following the use of active water samples, the tube is alternately evacuated and flushed eight times with inactive water vapor at about 17 mm. of mercury pressure, followed by a room air rinse and reëvacuation to 0.3 μ. This procedure has to be carried out after each activity measurement. An alternative method is the conversion of the labeled water to labeled hydrogen, which is then introduced into the counter.[21]

VII. Apparatus for Activity Measurements

A. GENERAL REMARKS

The radioactivity of biological samples is usually determined by means of a Geiger-Müller counting tube or with an electroscope.

[20] P. F. Hahn, *Ind. Eng. Chem., Anal. Ed.*, **17**, 45 (1945).

[21] R. Cornog and W. F. Libby, *Phys. Rev.*, **59**, 1046 (1941). N. Pace, L. Kline, H. K. Schachman, and M. Harfenist, personal communication.

[22] M. B. Allen and S. Ruben, *J. Am. Chem. Soc.*, **64**, 948 (1942).

Electrometers, especially of the Lauritsen type,[23] are often used but, in view of the greater sensitivity of the tube counter, this instrument is often preferred to the electrometer. An ionizing chamber–electrometer of the type used by Segrè, and by Amaldi and Fermi[24,25] is stated[26] to have a very high sensitivity, amounting to one-third to one-fifth that of the G-M counting tube when measuring the activity of weak samples of ^{11}C. This type of chamber–electrometer has a volume of 250 ml. containing nitrogen at 25 lb. gage pressure; its window, 8 cm. in diameter, is of 0.1-mm. aluminum foil connected to a single-fiber Lutz-Edelman electrometer. Such high pressure ionizing chambers obviously can not be used in the measurement of very soft rays, except when the sample may be placed inside the ionizing chamber. In the measurement of very soft rays, the sample may be placed inside the tube counter, for example in the tube devised by Libby[27] (see also page 71). Work with this type of counting tube is more exacting than that with a sealed type, which is therefore preferred (see page 60).

The relative advantages and disadvantages of the different measuring devices are discussed by Pollard and Davidson.[28] Each laboratory disposed, more or less, of its own counting arrangement. In the following discussion we shall describe a counting apparatus suitable for measuring small solid samples (up to a few hundred milligrams) and a glass counter used in the activity measurements of solutions. The former counter found extended application in the Institute for Theoretical Physics, University of Copenhagen, and in the Institute for Research in Organic Chemistry, University of Stockholm. The description which follows is that of Ambrosen, Madsen, Ottesen, and Zerahn.[29,30]

A detailed description of different types of G–M counters is given by Korff.[31]

[23] C. C. Lauritsen and T. Lauritsen, *Rev. Sci. Instruments*, **8**, 438 (1937.)

[24] E. Segrè, *Nuovo Cimento*, **12**, 237 (1935).

[25] E. Amaldi and E. Fermi, *Phys. Rev.*, **50**, 899 (1936).

[26] J. H. C. Smith and D. B. Cowie, *J. Applied Phys.*, **12**, 78 (1941).

[27] W. F. Libby and D. D. Lee, *Phys. Rev.*, **55**, 245 (1939).

[28] E. Pollard and W. L. Davidson, *Applied Nuclear Physics*, Wiley, New York, 1946.

[29] J. Ambrosen, *Acta Physiol. Scand.*, **10**, 205 (1945). J. Ottesen, *ibid.*, **10**, 201 (1945). K. Zerahn, *ibid.*, **10**, 209 (1945); **9**, 346 (1945).

[30] B. Madsen, *Acta Physiol. Scand.*, **10**, 195 (1945).

[31] S. E. Korff, *Electron and Nuclear Counters*. Van Nostrand, New York, 1946.

B. THE GEIGER-MÜLLER COUNTER[30]

For recording single α-particles, Rutherford and Geiger several years ago suggested an "electrical method." An electrical field with large gradient is formed between a wire and a plate. The electrodes are enclosed in a gas of suitable pressure. When an ionizing particle passes through the field it is possible by suitable choice of potential to obtain a secondary ionization due to collision, so that the voltage impulse is proportional to the amount of primary ions even though considerably amplified. Counters based on this principle are applicable to measurement of radiation of intense ionization, but not to measurement of β-particles.

In the G-M counter tube the ionizing particle is sent into a field with a large gradient, between a cylinder and a wire placed in the tube axis, but the voltage over the counter is chosen so as to exceed the limit of proportionality. As before, the ions are amplified due to collision, the factor of multiplication, however, being so great that the light of recombination forms photoelectrons in numbers sufficient to continue a stable discharge. If the voltage is reduced, the discharge can be extinguished and, for this purpose, the resistance, R, is inserted (Figure 1). Used in this way the counter responds to any ionizing radiation, giving, however, no information regarding the magnitude of the primary ionization. The counter operates most satisfactorily when, as is shown in Figure 1, the wire is made the anode.
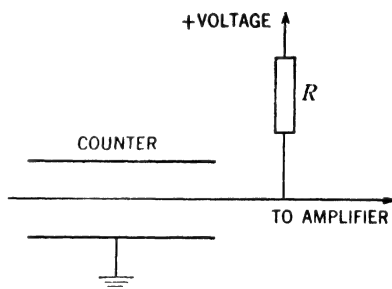
Fig. 1. Working scheme of a counter.[30]

If the filling gas is a pure gas (air or hydrogen), the space charge formed by the positive ions is of little influence and, to extinguish the discharge, the resistance, R, must have a value of about 10^9 ohms. This is inconvenient, for such high resistances do not keep their values constant, and further the time constant for the system counter capacity −series resistance is 10^{-2} to 10^{-3} sec. This quantity determines the resolving power of the counter. Small admixtures of heavy vapors, especially alcohol vapor, enable the ions to form a space charge sufficiently stable to extinguish the discharge. Then R can be made small, 1–10 megohms. Now the resolving time, τ, of the counter is a quantity associated only with the interior processes in the counter. Ordinary

gas filling gives a value of $\tau \approx 10^{-4}$ sec. for counters of normal size and still less for smaller counters. For the actual use of a counter, knowledge of the counting rate as a function of the voltage is necessary. At a certain voltage the counter starts recording; with increasing voltage, the number of pulses increases rapidly to a value which is more or less constant over a certain range of voltage until the number finally begins
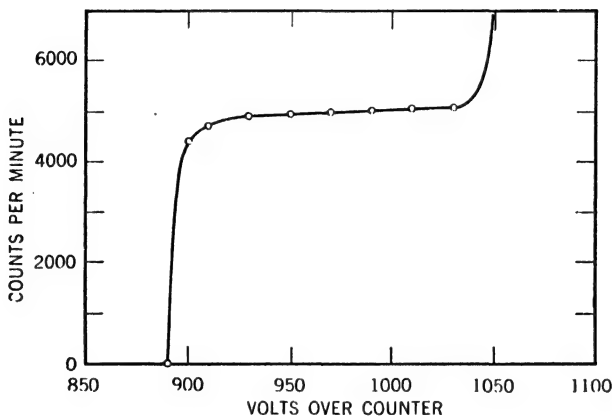

Fig. 2.   Plateau characteristics of a counter.[30]

to increase again. In Figure 2 the number of pulses is shown as a function of the voltage for a counter of the type described below.

It is possible to define "counting" (counting rate) only if the characteristic curve shows a constant range or plateau. In this case, counting is proportional to the intensity of primary radiation when losses due to the limited resolving power of the counter are taken into consideration. If a particle passes through the counter in a boundary domain of weak electric field it is not recorded with certainty. The number of pulses within counting range of the voltage will then depend somewhat on the voltage, so that a plateau in the characteristic curve may not be expected, especially for comparatively short counters.

When a counter discharge has taken place, there is a certain probability for the start of a secondary discharge not due to ionizing radiation penetrating into the counter. This probability increases with the overvoltage (= counter voltage -- starting voltage) and decreases rapidly with time. The secondary discharges afford the principal cause of the gradient of the characteristic curve. The magnitude of

the voltage pulses increases with overvoltage, the increase being greatest at the beginning of the characteristic curve. The starting voltage of the counter therefore depends somewhat on the sensitivity of the registering apparatus.

C. DETAILS OF CONSTRUCTION

The best counters are those filled with a mixture of 90 mm. argon and 10 mm. alcohol. Contamination by air within about 10% proved to have no essential effect. Further, the cathode material — but not the anode material — is of importance for the quality of the counter. Brass is generally used, but slightly oxidized iron is just as suitable. The secondary discharge effect can be somewhat diminished by gilding the inner side of the cathode.

With the use of counters for series measurements, often extended over a long time, stability and durability are important. This requirement, *inter alia*, makes it necessary to use clear materials for insulation and cementing. Counters built with ebonite insulators and cemented with picene are not durable, because these materials emit gases and absorb the alcohol vapor, whereupon refilling soon becomes necessary.

When the radiation to be measured is a penetrating radiation, it is preferable to build the counter enclosed in a glass vessel which, after gas filling, is sealed off. If, however, the radiation consists, as is usual in the work with radioactive tracers, of β-particles, a thin window is indispensable. Mica is used because of its considerable strength, and among many cements selenium has been found to be the most suitable. Other demands are: a large space angle and a small background count (zero count), so that a weak radiation can be measured. If no radioactive contamination is present, the background count is nearly proportional to the surface of the counter.

A handy construction is shown in Figure 3. On a tube of brass or compressed steel an edge is turned and over this a steel flange, A, is pressed. Between this and another steel flange, B, a mica window is cemented. Surrounding the tube, a cover of Jena glass (16^{111}) stands cemented upon A. The wire, a platinum wire 0.15 mm. in diameter, ending in a sphere about 0.6 mm. in diameter, is introduced through and supported by a glass tube. A slight improvement in the correct measurement of the relative counts of samples of greatly varying activities can be obtained by making the inner side of the mica window conducting; this can easily be done by burning gold

on the mica. Then the sphere on the counting wire must be placed
5–6 mm. from the window. During evacuation and filling, the side
tube, narrowed for sealing off, is connected with the pump arrangement
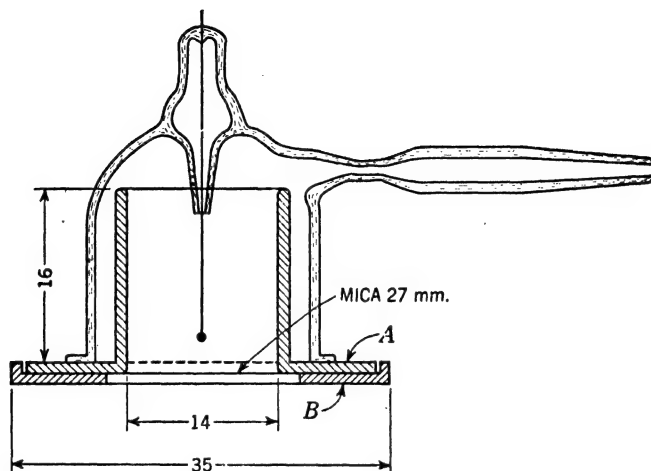by short, thick-walled rubber tubing. Between fillings with alcohol

Fig. 3. A counter.[30]

vapor and argon the connection is closed by a clip. The flange of the
glass cover is ground to a plane by means of carborundum powder.
After the flange is washed with water, the remaining grinding powder
is removed by etching with hydrofluoric acid. The mica window is
28 mm. in diameter. Windows weighing about 2 mg. per cm.^{2}, with a
free diameter of 14 mm., are used. Thinner windows will not safely
stand atmospheric pressure.

Before the counter is assembled it is necessary to remove any chance
of radioactive contamination. Glass, mica, and steel are wetted with
alcohol and plunged into concentrated nitric acid. Brass is rinsed in
dilute nitric acid. The counter is assembled on a heated brass plate
where a small crucible with selenium is also placed. One must avoid
use of too much selenium on flange A, since if so much selenium is used
that it runs over the rounded edge of the flange, the window is bent
sharply and may rupture. If any selenium vapor is condensed on the
glass cover it is removed by means of a small flame while the counter
is still hot.

After assembling, the counter must be cooled rather rapidly. During
slow cooling the selenium solidifies in a gray crystalline form, and the

glass-metal joint is probably not vacuum-tight. A suitable cooling rate is obtained when the counter is pushed from the hot plate to a cold brass block. The selenium then becomes smooth, black, and viscous, and even after cooling the counter to 0° the joint remains tight. The flanges are made of iron because of its small coefficient of expansion. Before the filling the counter must be tested for tightness. Small leaks may be difficult to discover. In the finished counter they disclose themselves by a uniform rise in the start potential.

This counter has an effective space angle of $\frac{1}{5} \times 4\pi - \frac{1}{4} \times 4\pi$. The preparation is placed close to flange B. If the counter as well as its surroundings are free from radioactive contamination, the background count can be reduced to about 2 counts per min. by shielding with 5–10 cm. lead.

The counter voltage is usually taken from a high-tension rectifying supply giving 2800 v. (d.c.); 1260 v. is laid over a filter consisting of resistances and condensers. The remaining 1540 v. is stabilized by means of 11 small neon lamps. This high-tension supply, which is much used in connection with G-M counters, gives a very constant voltage.

When using a very thin mica window, the counter described is suitable even for the measurement of the activity of rays as soft as those emitted by radiosulfur. The sensitivity of the counter was determined by Ottesen[32] by measuring the counts produced by a few (2–5) mg. U_3O_8 placed in an aluminum dish, 0.5 cm. in diameter, covered with a 0.015-mm. aluminum foil and fixed on a brass plate below the counter. It was found that 1 mg. U_3O_8 produced 236 counts per minute (which equals 38% of the β-particles emitted), the β-particles emitted by UX_2 being almost exclusively responsible for the number of counts registered.

It can be shown that, if no absorption in the counter window took place, the figure would be 265. Since 1 mg. U_3O_8 has an activity of 2.79×10^{-4} microcurie, a uranium preparation of infinitesimal weight having an activity of 1 microcurie would emit 9.50×10^5 counts per minute. When an aluminum dish 1.2 cm. in diameter is used the corresponding figure is 4.3×10^5. A sample of potassium chloride weighing 300 mg. was found to produce 35 counts per minute. Uranium standards, as mentioned above, are useful in checking the sensitivity

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[32] J. Ottesen, personal communication.

of the counter (which might change with time), and in comparing the data obtained by different counters.

D. AMPLIFIER AND RECORDER CIRCUIT

At normal working voltage the above-described counter produces impulses of the magnitude of about 0.5 v. Since the scale circuit described later required impulses of about 50 v. the amplifier must yield an amplification of more than 100 times. This amplification is most suitably obtained by the use of two stages.[33] As mentioned before, the counter is self-extinguishing and can therefore be connected with a usual proportional amplifier, even if a small leakage resistance is used. Nevertheless it has been found that in some cases it might be advantageous to make the resolving time of the amplifier, *i.e.*, the time that passes from the moment the amplifier has reacted to an impulse until it can again react to an impulse, somewhat larger than the resolving time of the counter. In this manner some of the secondary discharges will be avoided in counting. The probability that a discharge produced by an ionizing particle is followed by a secondary discharge increases considerably with the overvoltage and, in this way, a larger serviceable range of voltage and a smaller increase on the plateau characteristics of the counter can be obtained.

An amplifier with suitable resolving time is preferably obtained by means of a multivibrator circuit; with a double pentode of the same type as the scale valves (*e.g.*, the ELL1 type) a very simple construction is obtained (Figure 4). The impulse of the counter is transferred to the control grid of the first valve. Voltage divider $R_1 - (R_2 + R_4)$ and cathode resistance R_7 are adapted in such a way that the first valve amplifies reasonably; the anode current of the first valve is then about 3 ma. The grid bias for the second valve is chosen so that the anode current is nearly zero. A small negative impulse on the grid of the first valve will cause an increase in the anode potential of this valve. This increase is coupled to the grid of the second valve through condenser C_1 with the result that the anode potential of the second valve decreases. This decrease in turn is transferred to the grid of the first valve mainly through condenser C_2, etc. The process continues until the anode current in the first valve is zero and the anode and the cathode in the second valve are at the same potential. By means of voltage divider $R_1 - R_2$ the control grid of the first valve is

[33] J. Ottesen, *Acta Physiol. Scand.*, **10**, 201 (1945).

kept negative just as long as the anode voltage of the second valve is not altered. Therefore this state is steady until the potential of the control grid of the second valve decreases below the cathode voltage by charging condenser C_1. Then the anode potential of the second valve is increasing. By means of condenser C_2 this increase is trans-
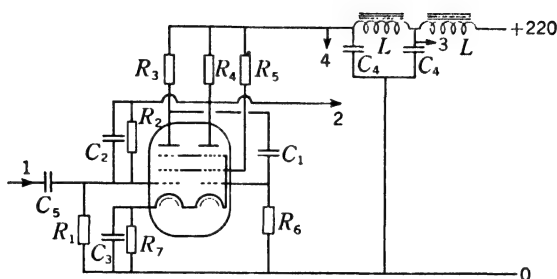
Fig. 4.   Amplifier.[33]

$R_1 = 0.05$ mΩ $C_1 = 100$–500 picofarads
$R_2 = 1.2$ mΩ $C_2 = 3$ picofarads
$R_3 = 0.03$ mΩ $C_3 = 10000$ picofarads
$R_4 = 0.05$ mΩ $C_4 = 16$ microfarads
$R_5 = 0.5$ mΩ $C_5 = 300$ picofarads
$R_6 = 0.1$ mΩ $L = 4$ henrys
$R_7 = 0.002$ mΩ

Terminal 1 is connected to the counter wire.
Terminal 2 is connected to "in" by the first
                    scale circuit.
Terminal 4 is positive voltage for the screen
            grid of the first scale circuit.
Terminal 3 delivers positive voltage for the
            rest of the scale circuit.

ferred to the grid of the first valve, etc. The currents in the valves very quickly resume the values which they had before the impulse arrived from the counter. The decrease in voltage of the anode of the first valve will produce a considerable negative voltage of the control grid of the second valve through C_1, so that for a short time after the process, a much larger impulse than usual is required to make the amplifier react. When C_1 has been discharged through $R_6 + R_3$ the amplifier will have completely recovered.

The resolving time of the amplifier is almost proportional to the capacity of condenser C_1. The resolving time of counter + amplifier is measured by means of two radium sources and a "scale-of-128" to 7×10^{-5} sec. with $C_1 = 100$ picofarads, the working voltage of the counter being 100 v. above the starting voltage. The method is described

by Ambrosen.[29]   The resolving time decreases a little when the voltage of the counter increases. With $C_1 = 100$ picofarads, and an overvoltage of 220 v. it has been measured to 5×10^{-5} sec.

The smallest resolving time which can be used is determined by the counter.   Many counters give a poor plateau and may be useless when the amplifier has a small resolving time; but with $C_1 = 500$ picofarads, most of the secondary discharges are not counted and consequently a good plateau is obtained.   As mentioned on page 59, the described counter joined together by selenium perhaps allows faster counting when the cathode is gold plated.
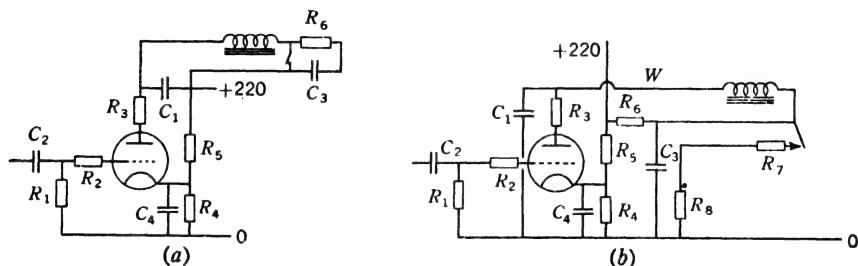
Fig. 5.   Amplifier.[34]

(a)

$R_1 = 0.5$ mΩ	$C_1 = 5000$ picofarads
$R_2 = 0.5$ mΩ	$C_2 = 100$ picofarads
$R_3 = 2$ kΩ	$C_3 = 0.1$ microfarads
$R_4 = 5$ kΩ	$C_4 = 2$ microfarads
$R_5 = 25$ kΩ	
$R_6 = 200$ Ω	

(b)

$R_1 = 0.5$ mΩ	$R_7 = 200$ Ω
$R_2 = 0.1$ mΩ	$R_8 = 200$ Ω
$R_3 = 500$ Ω	$C_1 = 100$ picofarads
$R_4 = 5$ kΩ	$C_2 = 100$ picofarads
$R_5 = 25$ kΩ	$C_3 = 1$ microfarad
$R_6 = 5$ kΩ	$C_4 = 2$ microfarads

The amplifier has been tested with four different valves, which all operated with an anode voltage supply varying from 180 to 235 v. The starting voltage of the counter is up to 15 v. lower at 180 v. than at 235 v.   At 210 v. the difference between the starting voltages was no more than 15 v.   If condenser C_2 is omitted the amplification will depend more upon the voltage and may fail at 180 v. with the resistance values used.

The anode supply is obtained from 220-v. (d.c.) mains. The noise

impulses are removed by means of two filters, each including a choke and an electrolytic condenser. The filament supply is obtained from 220-v (a.c.) mains by means of a transformer. Here, the noise impulses are greatly diminished by two condensers, 10,000 pf. each between primary terminal and earth. The rest of the noise impulses is eliminated from the cathode by condenser C_3. Shielded in this manner the amplifier only counts the noise impulses produced by switches close to the amplifier, *i.e.*, within a distance smaller than about 1 meter.

The mechanical recorder is a standard telephone message register. It is driven by a gas-filled triode. The discharge is quenched either by a "breaking" contact which interrupts the current or a "making" contact which decreases the anode potential to zero. In the circuits shown in Figures 4 and 5 the noise impulses produced by these processes are so much diminished that they do not interfere with the amplifier. In the circuit of Figure 5 this is mainly accomplished by condenser C_1 and resistance R_3.

E. SCALE CIRCUIT AND STATISTIC ANALYSIS[34]

The resolving time for the mechanical recorder is much greater than for the counter. At higher rates of counting it therefore becomes necessary to diminish the number of impulses before they reach the recorder. This is accomplished by inserting between amplifier and recorder a number of stages of the "scale-of-two." Each successive stage divides the counting rate by two. Figure 6 shows the principal features of such a system. It contains two electronic tubes with anode circuit resistances, R_2 and R_3, and grid-leak resistances, R_6 and R_7. A resistance and a condenser in parallel are connected from each anode to the grid of the opposite tube. One tube will allow anode current to pass, so that its anode potential is low. Accordingly the grid potential is low in the other tube, and will not allow any anode current to pass. Using suitable values of the components the system has two stable states with anode currents passing in one tube and with the other tube nonconducting. The impulses to be counted are impressed on the grids in such a way that every impulse causes the system to jump from one of the stable states to the other. The potential on one anode will be raised by every second entering particle and lowered by every other one; consequently the number of impulses transmitted to the next stage is only half the original number. In the diagram in question

[34] J. Ambrosen, *Acta Physiol. Scand.*, **10**, 205 (1945).

tubes of the type ELL1 are used; these contain two identical pentode systems placed side by side in a normal-sized bulb. This gives an easily wired and compact scale-of-two. The diagram with specifications below the figure is given in detail in Figure 7. The entering impulses have to be negative and must have a size of about 50 v. This rather large threshold value again assures that the circuit is not easily affected by electrical disturbances. It is possible to use a series of stages one after the other, all containing identical components.
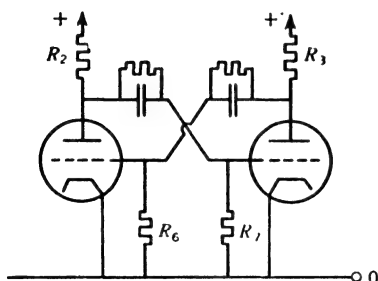
Fig. 6. Principal features of a
scale-of-two counter.[34]

R_1, R_6, and $R_7 = 50$ kΩ
R_2 and $R_3 = 50$ kΩ (2 w. at least)
R_4 and $R_5 = 100$ kΩ
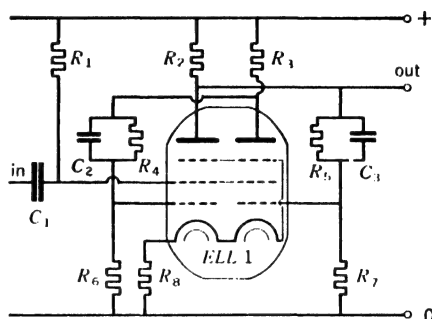
Fig. 7. Diagram of a scale-of-two
counter.[34]

$R_8 = 3$ kΩ (variable)
$C_1 = 100$ picofarads
C_2 and $C_3 = 300$ picofarads

Correct functioning is ensured by adjusting cathode resistance R_8; if this is too high, the system will be insensitive, and if it is too low, the tubes will oscillate between the two stable states at a very high frequency. The application of one scale-of-two has the effect that the fraction of particles lost is unaltered although the register now counts 7.5 times faster. This improvement is caused by the altered distribution in time of the impulses transmitted to the register.

F. ARRANGEMENT FOR AUTOMATIC MEASUREMENT

In radioactive tracer work a frequent task is the comparative measuring of radioactive samples. It is not specially troublesome work, but it requires someone to change the samples and to read the mechanical counter. When many samples are to be measured, it may be of importance to do this automatically.[35] An arrangement answering this purpose is described in the following. The samples to be measured are

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[35] K. Zerahn, *Acta Physiol. Scand.*, **10**, 209 (1945).

placed in small aluminum dishes. The samples are changed when a clock makes a contact and each sample is measured on its own mechanical recorder. Figure 8 shows the apparatus in cross-section and Figure 9 as it is seen from above. The aluminum dishes are placed in the corners of a regular hexagon. The disc is firmly fixed to a shaft, A, which can move on two pin-point centers.

Since the counting rate is dependent on the distance from the sample to the G-M counter, S, must not warp more than a fraction of 0.1 mm. Shaft and disc are moved by a weight of about 1 kg. suspended in a steel wire (not shown in Fig. 8). The steel wire is wound on a pulley, T_1 which is fastened to another pulley, T_2. On T_2 another steel wire is wound in the direction opposite to the first wire, holding a small weight. The pulleys are fastened to a ratchet wheel, R, and can therefore turn freely about the shaft in one direction. By pulling down the little weight, the apparatus can be prepared for a fresh set of counts. In the border of the disc, 30° from each indenture, a steel pin is fastened by a small machine screw. A pin, P, will stop the movement of the disc by pushing against a stop, KL, which is mounted on the anchor of a small electromagnet, M. One pin only can pass the stop when the anchor is attracted by the magnet.[35] The anchor is attracted when a synchronous clock connects two contact springs, e.g., every half hour. In order to avoid shaking the samples one has to moderate the movement of the disc; this may be done by connecting it with a piston moved in oil. Furthermore, an insulated radius, B, fastened to the shaft, carries a screw, K, which presses contact springs F together (only one couple is shown in Fig. 8). These contact springs connect the electrical circuit to the respective mechanical recorder. In Figure 8 a conducting wire is connected by the contact springs. The radius with the screw has to be placed on the shaft in such a way that the screw connects a couple of contact springs when a corresponding indenture is directly under the G-M counter. This position must be taken up when a pin is stopped by KL. The G-M counter is placed at C (Fig. 8) and can be surrounded by a thick layer of lead. Mechanical recorders with "making contacts" are used because they are most convenient.

When placing the radioactive samples in aluminum dishes and measuring them by pushing the dishes under a G-M counter, only a fraction of the rays is utilized. If the rays are not too weak, a greater part of the rays can be utilized by placing another counter under the aluminum dishes (see Fig. 8). Each counter is connected with an
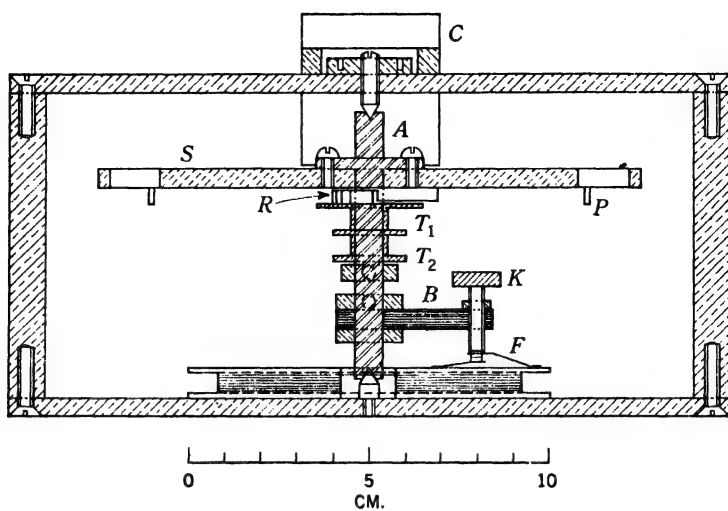
Fig. 8.   Arrangement (cross section) for automatic measurements of activity.[35]
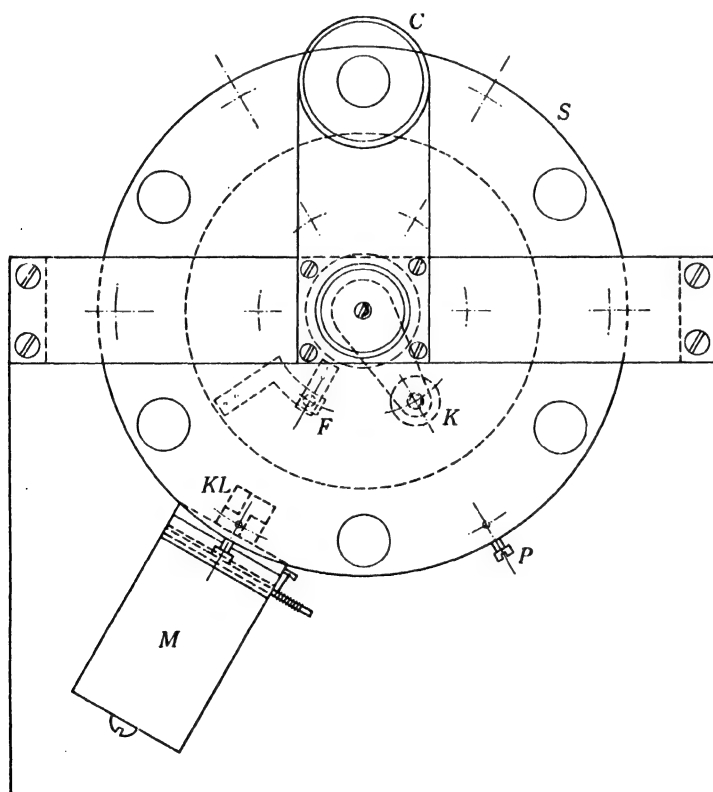
Fig. 9.   Arrangement (top view) for automatic measurements of activity.[35]

amplifier of its own. Thus the sample count will be recorded by the upper counter after absorption of some rays by the mica window and the air. With the lower counter the absorption due to 0.1-mm. aluminum (the thickness of the dish) will be added. If the rays from the sample are relatively hard the two counters will yield records of the same number, but if some of the rays are weak the upper counter will record relatively more. The use of two counters gives the following advantages: in the first place, two measurements of the sample are simultaneously obtained if the rays are not especially weak; in the second place, the ratio between the results from the upper and the lower counter makes it possible to estimate the hardness of the rays from the sample and so perhaps to detect the presence of false activity; finally, the statistical uncertainty will be diminished, because the total number of recorded impulses is increased.

Another automatic sample changer used for measuring radioactivity of samples was recently published by Peacock and Good.[36]

G. MICRO COUNTING ARRANGEMENT

In using the apparatus[37] to be described, dishes having a diameter of 5 mm. and a depth of 0.5 mm. are employed. The collar of the dish has a breadth of 3 mm. The dish is placed in the brass ring in *A* (Fig. 10), which fits the sledge used in all counting arrangements in the author's laboratory. To protect the sample an aluminum foil having a diameter of 14 mm. and a thickness of 0.01 mm. is pressed to the collar of the dish. The foil is pressed to the dish in the following way: the dish is placed on the brass holder (*B*, Fig. 10) and, after placing the sample into the dish, the foil is pressed with two fingers against the collar of the dish. The fingers used to carry out this process are protected by rubber coatings. The final fixing of the foil is carried out by placing the dish in the brass ring mentioned above and by pressing the foil with the cylinder (*C*, Fig. 10). We obtain the weight of the sample by weighing the dish after the aluminum foil is fixed and by determining the weight of the empty dish and the foil.

If we wish to know the activity of sample in percentage of radiophosphorus injected, a few cubic millimeters of the active solution is placed in an aluminum dish with the aid of a micro pipet. Thus a standard preparation is obtained. The dish is then treated as described

[36] W. C. Peacock and W. M. Good, *Rev. Sci. Instruments*, **17**, 255 (1946).
[37] J. Ottesen, *Acta Physiol. Scand.*, **10**, 214 (1945).

above. Although the weight of the standard preparation obtained by
the last-described treatment is negligible, the weight of a sample con-
taining nucleic acid, for example, may amount to several milligrams.
In such a sample some absorption of the β-rays emitted by the active
nucleic acid takes place. The error due to this absorption is determined
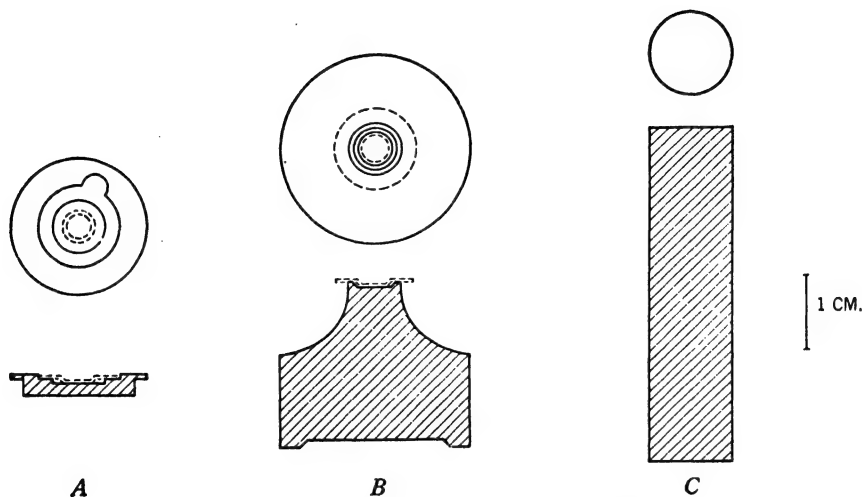
Fig. 10. Micro counting arrangement.[42]

as follows: the activity of a standard sample of negligible weight is
measured. The measurement is then repeated after placing 4 mg.
inactive nucleic acid above the standard sample. The activity is found
to be reduced by 8%. Assuming the absorption in a very thin layer of
nucleic acid to be proportional to the thickness of the layer, we find
the average activity of the nucleic acid sample weighing 4 mg. to be
4% smaller than the activity of a sample of negligible weight.

It is of interest to note that this error is to a large extent compen-
sated by the fact that by increasing the weight of the sample, the sample
comes nearer the window of the counter tube and produces a corre-
spondingly greater effect on the counter. The apparatus described is
more sensitive than that described on page 44. When measuring the
activity of small samples, *e.g.*, 2 mg., the number of counts registered
by the micro counter arrangement is at least one and one-half times as
high as that registered by the macro apparatus.

In measurements of very soft rays counters filled with helium or
helium and alcohol operated at atmospheric pressure can be used with

advantage. The use of such tubes makes it possible to employ very thin windows on the counter, since no pressure differential need be supported. Counters in which the sample is inserted inside the vacuum and moved into position in front of a wire screen window by a magnet[38] are also applied in the measurement of very soft radiation, for example, in the measurement of the activity of radioiron.[39]

H. THE GLASS COUNTER

Counting arrangements containing a glass counter introduced by Olson and colleagues[40] and by Bale and associates[41] have found extended application in tracer work. The following description of an improved
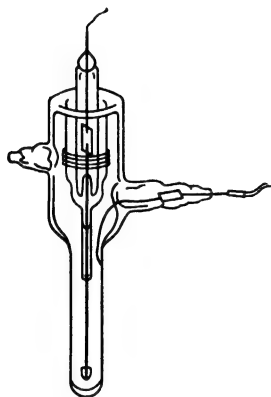
Fig. 11
Dipping-type
G-M counting tube.[42]
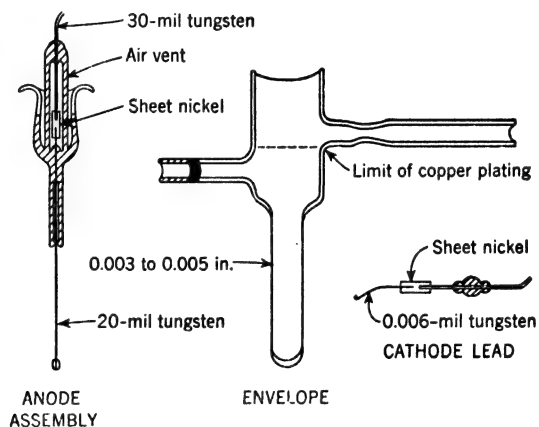
Fig. 12. Construction details of dipping-type tube.[42] Clear section, Pyrex glass; shaded section, Nonex glass; black section, cobalt glass.

type of glass counter has been reported by Bale and Bonner.[42] A cross section of the tube is shown in Figure 11.

The envelope is constructed of Pyrex glass except for the tungsten leads which are sealed in Nonex. The center wire, of 20-mil tungsten, is straightened by heating under tension *in vacuo*. A white heat for five or ten seconds is sufficient to straighten

[38] W. F. Libby and D. D. Lee, *Phys. Rev.*, **55**, 245 (1939).

[39] W. C. Peacock and W. M. Good, *Rev. Sci. Instruments*, **17**, 255 (1946).

[40] A. R. Olson, W. F. Libby, F. A. Long, and R. S. Halford, *J. Am. Chem. Soc.*, **58**, 1313 (1936).

[41] W. F. Bale, F. L. Haven, and M. L. Le Fevre, *Rev. Sci. Instruments*, **10**, 193 (1939).

[42] W. F. Bale, and J. F. Bonner, in A. Weissberger, *Physical Methods of Organic Chemistry*, Vol. II. Interscience, New York, 1946.

all except badly bent pieces of wire. Prolonged heating results in brittleness due to recrystallization of the tungsten fibers. The tube is constructed in three parts, as shown in Figure 12. Sheet-nickel intermediates are spot-welded to two 30-mil tungsten leads. A length of straightened 20-mil tungsten wire is welded to one of these, and is used in the anode assembly. A short piece of 6-mil wire is welded to the second lead, and is bent to make a spring contact with the cathode when sealed into the side arm.

The envelope is coated on the inside with copper by reduction of ammoniacal copper acetate with hydrazine hydrate. Two solutions are needed: (A) Cupric acetate, 30 grams per liter. Concentrated ammonium hydroxide is added until the precipitate of cupric hydroxide is just redissolved. pH, approximately 10.7. (B) Hydrazine hydrate, E. K. C., 85% in water. The envelope is cleaned by soaking it in cleaning solution overnight, and is thoroughly rinsed with distilled water. Sufficient plating solution to fill the tube is then mixed, 20 parts of A and one of B. The envelope containing the solution is immediately placed in a water bath at a temperature of 95° to 100° C. A film of the correct thickness will form in about one minute. If left in the bath too long, the film will be stripped from the glass by formation of bubbles under the film. The tube is left in the bath no longer than necessary, the plating solution is poured out quickly, and the tube is rinsed five or six times with distilled water, followed by acetone or alcohol–ether. If the film is too thin, or has started to peel, it may be removed with nitric acid and the process repeated. A little practice will give good films on the first trial.

The cathode lead is then sealed into the side arm, and a dab of Aquadag placed on the point of contact. To aid in centering the anode, the copper film is removed from the thickened end of the tube by rubbing with a stick tipped with moist cotton and a little fine abrasive (#400 Alundum). The anode assembly and the envelope are washed carefully with ether to remove all traces of grease and dust from the interior of the counter, and are then sealed together. The thickened end of the counting tube acts as a lens and makes it possible to center the anode quite accurately.

Immediately following assembly, and before sealing to a vacuum system, the tube is evacuated to remove moisture (from the glass blowing) and to test for failures. The tube is then sealed to the vacuum system and evacuated. It is filled with a mixture of 90% hydrogen and 10% absolute ethyl alcohol vapor to a total pressure of ten centimeters, tested with a countercircuit, and sealed off.

A 2-ml. aliquot of the solution in which the activity determination is to be made, or the whole sample made up to 2 ml. is placed in a glass cup, the inside diameter of which is about 1 mm. greater than the outside diameter of the counting tube, which is mounted as an inverted plunger. The cup is mounted on a slide which brings the cup up around the counter end to a point at which the liquid forms a 1-mm. thick film surrounding the sensitive area of the counting tube. (Such geometric conditions provide a good opportunity for β-rays to enter the G-M tube without traveling through an excessive amount of material, producing an apparatus of high sensitivity.) By using equal volumes of solution and by raising the slide bearing the cup to the same point against a mechanical stop, the same geometric conditions are reproduced for each sample and the resulting counts per minute are proportional to the radioactivity of each sample.

For the process of changing samples, a 25-ml. syringe containing distilled water, another containing acetone, and some absorbent cotton are useful. The sample is lowered and removed from the counting tube. A beaker is set on the sample holder, and the G-M tube sensitive portion washed by a fine stream of distilled water from a syringe. The washings are caught in the beaker. The tube is then gently wiped by a dampened tuft of absorbent cotton.

The dipping counter described is stated to have a background of the order of twelve counts per minute. The tube is available commercially (*Distillation Products, Inc.*, Rochester, New York). Glass counters may be affected by light and should be protected from intense illumination. All counters should preferably be provided with a light-tight box so that they operate in darkness.

Since the counts registered depend on the density of the solution, when comparing the activities of two solutions, appreciable density differences should preferably be avoided. The effect of the density of the solution on the number of counts[43] is seen in Table 12, in which the activity of the same aliquot of a ^{32}P sample dissolved in aqueous sulfuric acid solutions is recorded.

TABLE 12

Effect of Density Change of Solution of ^{32}P on Counts Recorded by the Glass Counter[43]

H_2SO_4, %	Density	Number of counts	H_2SO_4, %	Density	Number of counts
0	1	1000	70	1.615	650
10	1.069	900	80	1.733	560
30	1.221	776	90	1.819	491
50	1.399	721	95	1.839	498
60	1.504	660			

From the β-ray absorption curves with aluminum,[44] it follows that, from a given source in aqueous media, about 48% of the β-rays of ^{32}P are absorbed in each 0.1 cm. distance.

Bale and Bonner[42] with the dipping counter determined the activity of ^{32}P in the presence of appreciable amounts of bone salt. In these experiments increasing amounts of tricalcium phosphate were dissolved in equal aliquots of an acid solution containing radiophosphorus and the resulting activity was determined. By plotting the ratio of the

[43] A. Forssberg, personal communication.
[44] Chien-Shiung Wu, *Phys. Rev.*, **59**, 481 (1941).

calculated to the observed activity as ordinate against the weight of tricalcium phosphate present in the sample, a curve was obtained showing the ratio between theoretical and observed counts for any given amount of solute. With this counting tube, the relation was approximately linear and represented a 5% reduction in net counts on a 2-ml. aliquot containing 0.5 g. of tricalcium phosphate.

One may also obtain this absorption correction with a solution of unknown composition by adding a known activity of a highly radioactive preparation of the same isotope to the unknown material after its radioactive count has been made, and determining the difference in the two counts. The apparent percentage decrease in the activity of the last added component is the correction factor to apply to the net count of the original solution. With either of the above methods, appropriate allowances must be made for any change in solution volumes caused by the addition of the solute.

VIII. Measurement of Radiation Emitted at Points External to the Body

In the application of radioactive indicators in biological research we usually investigate tissues removed from the animal or plant organism after death or biopsy. Such procedure is largely indispensable since we must in many cases secure and purify some of the radioactive compounds present in the tissues. In some cases, however, we can measure, at points external to the human body, the radiation emitted by a radioactive substance taken up by a definite region of the body. The success of measuring such radiation will depend on the penetrating properties of the radiation and on the degree to which the radioactive isotope is localized in the tissue in question.

Hamilton at an early date measured the γ-radiation emitted by the hand of human subjects at various times after administration of radioactive ions of sodium, chlorine, bromine, and iodine (see page 95). Gamma-ray-emitting isotopes of elements which are localized to a high degree are the most suitable ones for such determinations. The accumulation of radioiodine which is localized to a large extent in the thyroid was followed by placing a G-M counter tube against the gland. The counter tube employed was 10 cm. long and 1.5 cm. in diameter; for mechanical protection it was encased in a copper tube whose walls were 1 mm. thick.

The study of the *in vivo* concentration of β-ray-emitting isotopes is

limited to the most superficial layers of the body, on account of the low penetration of the radiation. Their study is facilitated, on the other hand, by the fact that localization of the region is easily accomplished by light screening.
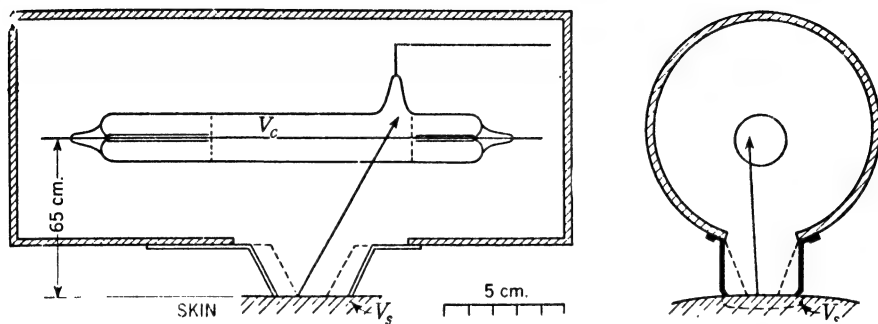
Fig. 13. Arrangement of G-M counting tube with housing and diaphragm, for measurement of body surface activity.[45]

Marinelli et al.[45] by means of a G-M counter studied *in situ* the deposition of ^{32}P in the superficial tissues of patients undergoing treatment with radiophosphorus. The arrangement they used is seen in Figure 13. The G-M tube counter provided with cylindrical glass walls 0.4 mm. thick and coated internally with silver was mounted in a cylinder with iron walls of about 3-mm. thickness, sufficient to cut out all β-radiation from ^{32}P. A rectangular window, 7×3 cm., was cut in the iron tube and was covered with black paper to exclude light, to which the counter is sensitive. Two brass diaphragms, 6.6 and 1.1 cm.² in area, were used. They were mounted on brass plates so that they could be placed by sliding in front of the window, in reproducible position and in contact with the skin of the patient. A scale drawing of the assembly is presented in the figure. The larger diaphragm is shown in place and the smaller one is indicated by the dotted lines. In the figure, V_c is the effective volume of the counter; V_s is the volume of skin from which β-rays can be counted.

The shape of the diaphragms permits every point of the contact surface to "see" all the effective volume of the counter. It was thought that with this precaution the counter readings would be proportional to the area of the diaphragm when placed over an extended source of homogeneous concentration. Experiment, however, proved the con-

[45] L. D. Marinelli and B. Goldschmidt, *Radiology*, **39**, 454 (1942).

trary. This was shown to be due to the difference in the average length of path traveled by the β-particles through the walls of the counter and the layers of the radioactive source. The ratio of the counting rates for the two diaphragms was found to be 4.9 with very thin sources and 5.4 with thick sources (thicker than the range of the ^{32}P β-particles). Further tests with aluminum filters yielded results in qualitative agreement with this view and experiments performed directly on the patients' skin confirmed the thick-source ratio. Strajman[45a] recently described a small counter for measurements *in vivo*.

When in operation, the counter was held by suitable clamps attached to an old x-ray tube stand. Proper counterweighting was provided to prevent excessive pressure on the patient. The latter was instructed to press slightly against the diaphragm if the pressure relaxed during the course of observation; this, it was found, was sufficient to ensure satisfactory reproduction of the readings.

IX. Accuracy of Counting Measurements

The conversion of one atom, say, radiophosphorus into stable sulfur, takes place at random; only a very large number of such events, when averaged, will give an accurate picture of the event. We must count numerous particles when, for example, we wish to compare the activity of two ^{32}P samples with appreciable accuracy. The probable error in counts associated with the total number of counts in a given period is given by the equation:

$$E_n = 0.67 \sqrt{N}$$

where N is the total number of counts observed.

Let us assume that 1000 individual counts have been recorded over a period of ten minutes. Substituting 1000 in the above formula, $E_n = 0.67 \sqrt{1000} = 21.4$. The percentage probable error works out to be $(21.4/1000) \times 100 = 2.14$.

When measuring 1000 individual counts over a period of ten minutes or 100 counts per minute, there is a 50% probability that the activity measured is within 2.14% over the genuine activity, *i.e.*, the activity which a measuring for an infinite period would supply. The number of counts which keeps the statistical fluctuations below a certain percentage is seen in Table 13.

[45a] E. Strajman, *Rev. Sci. Instruments*, **17**, 232 (1946).

When measuring small samples the background of the counter should be known with a similar accuracy as the activity of the sample. We measure, for example, 2500 counts when the aluminum dish contains the sample and the same number with an empty aluminum dish. Each

TABLE 13

Probable Error Involved in Counting

Percentage error	Number of counts
1	4445
2	1114
5	177
10	45
20	11

of these counts will have a 2% statistical uncertainty. Assuming the time of measurement to amount to 400 and 1000 minutes, respectively, we obtain for the activity of the preparation 6.25 ± 0.12 impulses per min. and for the background 4.17 ± 0.08. The net count for the preparation is then:

$$2.08 \pm \sqrt{0.12^2 + 0.08^2} = 0.145$$

The uncertainty of measurement thus is $\pm 7\%$.

A detailed discussion of the daily variation in background of counters is found in a paper by Evans.[46]

X. Photographic Technique

The photographic technique in tracer work is of great use when the radioelement administered is to be localized or the uptake of radioactive isotopes by the animal or plant organism is to be demonstrated. The difference in the uptake of radiophosphorus and radiostrontium by the skeleton, for example, or of radiogold by the cortex and the marrow of the kidneys is demonstrated strikingly in the radiograph shown in Fig. 38, page 184 (cf. also Fig. 39). Beautiful radiographs of sections of the thyroid which had taken up radioiodine were also taken (see page 143). In the same way the photographic technique was applied to demonstrate the presence of radium in the bones of a patient who died following the ingestion of large quantities of radium chloride.

[46] R. D. Evans, *J. Applied Phys.*, **12**, 342 (1941).

In the work on intermediary metabolism of the various elements, the application of the photographic technique is, however, restricted. The photographic action of an element is independent of the chemical composition in which the element is present. Inorganic phosphorus and phosphatide phosphorus, for example, show the same photographic action. Furthermore, the quantitative evaluation of radiographs often encounters difficulties.

Hamilton and his associates[47] studied the deposition of radioiodine in the thyroid gland of patients suffering from various types of goiter by using the photographic technique. Sections from 3 to 5 μ thick were prepared from the extirpated thyroid tissue by the usual paraffin impregnation technique. The sections were mounted on large glass slides and the paraffin was removed by washing in xylene. After the xylene had evaporated, the sections were dipped into dilute celloidin and set on edge to dry. The celloidin forms a thin film approximately 1 μ thick which serves to protect the sections. Each section was covered with a piece of Agfa "No-Screen X-Ray Film" which was firmly fastened with tape. The slides were stacked together and placed in a small press in order to hold the films in close contact with the sections. Several of the slides were wrapped individually and every two or three days one of these was opened and the film was developed in order to ascertain when the optimum degree of exposure had occurred.

For optimum results by this method, the films should be exposed from five to eight days with 3-μ sections cut from thyroid tissue containing 0.3 microcuries radioiodine per gram. This indicates that a total of approximately 2,000,000 β-particles must strike each square centimeter of the emulsion on the film in order to produce a satisfactory image. At least 0.15 microcurie radioiodine per gram of tissue must be present if suitable radioautographs are to be obtained from 3-μ section, but with this amount of exposure at least three weeks is required. An exposure of more than three weeks is of little value since at the end of this time more than 80% of the radioiodine in the sections has decayed.

Two precautions must be observed in order to secure the optimum degree of resolution. First, the sections should not be thicker than 10 μ, and second, the celloidin film covering the sections must not exceed 2 μ in thickness. The sections should be of uniform thickness and should

[47] J. G. Hamilton, M. H. Soley, and K. B. Eichorn, *Univ. of Calif., Pub. Pharmacology*, **1**, No. 28 (1940). See also L. F. Bélanger and C. P. Leblond, *Endocrinology* **39**, 8 (1946).

be mounted on slides without wrinkling. Hamilton and associates have administered to patients radioiodine samples the radioactivity of which varied between 100 and 1000 microcuries.

The β-ray spectra of radioiodine (^{131}I) have an upper energy limit of 690,000 e.v., but the energy of the majority of the β-rays lies between 250,000 and 100,000 e.v. The upper energy limit of β-rays emitted by ^{32}P is 1.7 m.e.v. and the average energy of the emitted particle is 700,000 e.v. When making radiographs of sections containing ^{32}P the sections may be correspondingly thicker, as stated above. The intensity of radiographs can be much enhanced by prolonged exposure of the photographic film to the effect of the radiation. Bone slices weighing 100 mg. and containing radiophosphorus emitting 500 β-particles per minute produce radiographs of remarkable intensity only when acting for three weeks on a photographic film.[48] Eastman "No-Screen X-Ray Film" was found to be very suitable to take radiographs of leaves bearing a radioactive carbon content, showing an activity of 225 disintegrations per minute only.[49] A list of the average energies of various radioactive isotopes is given by Marinelli *et al.*[50]

[48] O. Erbacher, *Z. angew. Phot. Wiss. u. Tech.*, **1**, 141 (1939).

[49] A. V. Grosse and L. C. Snyder, *Science*, **105**, 240 (1947).

[50] L. D. Marinelli, R. F. Brinckerhof, and G. J. Hine, *Revs. Modern Phys.*, **19**, 25 (1947).

XI. Decay of Activity of Radiosodium, Radiopotassium, and Radiophosphorus

TABLE 14

Decay of Activity of ^{24}Na (Half-life = 14.8 Hours)

Hours	Activity	Hours	Activity	Hours	Activity	Hours	Activity
0	10000	19	4107	38	1687	66	455
1	9542	20	3919	39	1610	68	414
2	9106	21	3740	40	1536	70	377
3	8689	22	3569	41	1466	72	343
4	8292	23	3406	42	1399	74	313
5	7912	24	3250	43	1335	76	285
6	7550	25	3101	44	1274	78	259
7	7205	26	2959	45	1215	80	236
8	6875	27	2824	46	1160	82	215
9	6561	28	2694	47	1107	84	196
10	6260	29	2571	48	1056	86	178
11	5974	30	2454	50	962	88	162
12	5701	31	2341	52	876	90	148
13	5440	32	2234	54	797	92	135
14	5191	33	2132	56	726	94	123
15	4953	34	2034	58	661	96	112
16	4727	35	1941	60	602	98	102
17	4510	36	1853	62	548	100	92
18	4304	37	1768	64	499		

TABLE 15. Decay of Activity of ^{42}K (Half-life = 12.4 Hours)

Hours	Activity	Hours	Activity	Hours	Activity	Hours	Activity
0	10000	25	2475	50	611.3	75	151.2
1	9460	26	2337	51	578.4	76	142.9
2	8946	27	2213	52	546.8	77	135.2
3	8452	28	2092	53	516.8	78	127.8
4	8000	29	1976	54	489.0	79	120.8
5	7563	30	1869	55	462.3	80	114.3
6	7148	31	1770	56	436.9	81	108.1
7	6761	32	1672	57	413.2	82	102.2
8	6394	33	1582	58	390.8	83	96.63
9	6046	34	1497	59	369.5	84	91.42
10	5717	35	1415	60	348.8	85	86.42
11	5407	36	1337	61	330.7	86	81.70
12	5119	37	1264	62	312.8	87	77.28
13	4833	38	1196	63	295.5	88	73.10
14	4575	39	1131	64	279.6	89	69.10
15	4325	40	1070	65	264.4	90	65.36
16	4086	41	1012	66	249.9	91	61.84
17	3865	42	956.0	67	236.4	92	58.44
18	3656	43	904.3	68	223.5	93	55.25
19	3458	44	855.4	69	211.3	94	52.28
20	3269	45	809.2	70	199.9	95	49.43
21	3096	46	764.5	71	189.1	96	46.71
22	2924	47	723.2	72	178.8	97	44.19
23	2763	48	683.4	73	169.0	98	43.61
24	2618	49	646.4	74	159.9	99	39.48
						100	37.37

TABLE 16. Decay of Activity of ^{32}P (Half-life = 14.30 Days)

Days	Activity	Days	Activity	Days	Activity	Days	Activity
0	10000	14	5073	40	1439	66	408
1	9527	16	4604	42	1306	68	370
2	9076	18	4179	44	1185	70	336
3	8647	20	3793	46	1076	72	305
4	8238	22	3443	48	976	74	277
5	7848	24	3125	50	886	76	251
6	7476	26	2836	52	804	78	228
7	7123	28	2574	54	730	80	207
8	6786	30	2336	56	662	82	188
9	6465	32	2120	58	601	84	171
10	6159	34	1924	60	546	86	155
11	5867	36	1747	62	495	88	140
12	5590	38	1585	64	450	90	127
13	5325						

Atomic Interchange

I. General Remarks

None or only few readers of this volume will remember the fierce discussion raging for many years over Arrhenius' conception of electrolytic dissociation. Had isotopic indicators been available during the last quarter of the nineteenth century, the issue could have been easily decided. If salts, for example, lead chloride and lead nitrate, are dissociated in solution, as postulated by Arrhenius, the following should be true upon mixing equimolar amounts of normal lead chloride and labeled lead nitrate in aqueous solution and allowing the lead chloride to crystallize again, the active lead atoms should be distributed proportionally between chloride and nitrate, having migrated in solution from lead nitrate to lead chloride until kinetic equilibrium had been established. This was actually found to be the case. Similar results are obtained when lead chloride and lead are dissolved in pyridine. A very different behavior, however, was observed in all cases in which the lead atom was linked to carbon. No exchange of lead atoms could be established between lead chloride and tetraphenyllead in pyridine, between lead nitrate and tetraphenyllead in amyl alcohol, and between lead nitrate and diphenyllead nitrate in aqueous ethyl alcohol, one of the partners involved in the crystallization process being an organic, non-dissociated compound.[1] No interchange of lead atoms was found to take place in strongly alkaline plumbite and plumbate solutions,[2] these solutions not being dissociated to any appreciable degree.

The question of to what extent atoms incorporated in organic molecules interchange with atoms present in other molecules or ions is of greatest importance for the applicability of isotopic indicators in biological research. We use isotopic tracers in the determination of rate and place of formation of biochemically important compounds in the organism and try to elucidate the mechanism of the enzymic processes

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[1] G. Hevesy and L. Zechmeister, *Ber.*, **53**, 410 (1920).
[2] E. Zintl and A. Rauch, *Ber.*, **57**, 1739 (1924).

leading to the formation of compounds such as phosphatides, nucleic acid, glycogen, methionine, and so on. All these determinations are based on the assumption that the tracer atoms, ions, or radicals do not interchange with the corresponding constituents of the molecules in question. The administered labeled phosphate is assumed not to exchange with the phosphate radical of the phosphatide molecule, for example, by any simple "physical" exchange process. The formation of labeled phosphatides by collision between an inorganic phosphate molecule and a phosphatide molecule solely as a result of such a collision is assumed not to take place. The formation of labeled phosphatide molecules in the organism takes place as a result of an enzymic process which is coupled with an energy-producing system. Important evidence that the formation of labeled phosphatide is not due to exchange through collision between the labeled phosphate and nonlabeled phosphatide molecules or between intermediary products of the formation of phosphatide molecules, was presented by Chaikoff *et al.* In their experiment with liver slices (see Chapter VIII) they found that the formation of labeled phosphatides does not take place under anaerobic conditions or in the presence of respiratory inhibitors. The results also exclude the possibility that the formation of labeled phosphatides is due to a reversibility of their degradation.

A single chemical reaction is usually considered the net result of two opposing reactions proceeding simultaneously in opposite directions at different rates. Even though the tendency of the over-all reaction is far in the direction of decomposition (degradation of phosphatides), the reverse reaction may occur to a slight degree. This reversibility could result in replacement of the phosphate of the phosphatides by labeled phosphate without the addition of energy from energy-producing reactions. This type of exchange can, however, also be excluded as a result of experiments of Chaikoff *et al.* They found that respiratory inhibitors block the formation of tagged phosphatides in tissue slices incubated in Ringer solution containing labeled phosphate. The presence of respiratory inhibitors, while inhibiting the energy-producing reaction indispensable for the formation of labeled phosphatides, should clearly not interfere with a reversibility of the above-mentioned type.

Since in a fully grown organism the phosphatide content of the organs is about constant, the formation of new (labeled) phosphatide mole-

cules must proceed simultaneously with enzymic degradation or removal of an approximately equal number of "old" molecules. The determination of the percentage formation of labeled molecules thus indicates the percentage degradation (or removal) of the molecules originally present as well.

That a "physical" exchange of the phosphate group of the phosphatides and other organic phosphorus compounds present in the organism does not take place is shown by shaking a solution of such compounds with labeled phosphate and determining whether the organic compound became radioactive. After shaking a solution of hexose monophosphate,[3] glycerophosphate,[4] lecithin, casein,[5] or nucleic acid[6] with a solution of labeled phosphate, no organic labeled phosphorus was found to be present. Nor was interchange found to take place between the phosphorus atoms of orthophosphate and the phosphorus atoms of pyrophosphate and metaphosphate,[7] or phosphorus and phosphoric acids in acid or alkaline solution, or between phosphate and hypophosphite in neutral or acid solution.[8]

No exchange takes place between the sulfur atoms of cysteine, thioglycolic acid, or thiourea and those of H_2S, S^{2-}, or SH^-, respectively,[9] nor when elementary sulfur is dissolved in carbon disulfide and the solution is kept at 100° for 68 hours.[10] No exchange interaction was found to take place between sulfide and sulfate or between sulfite and sulfate.[11] Experiments carried out for 36 hours at 100° gave an entirely negative result.[11] The two kinds of sulfur atoms present in the thiosulfate molecule were found not to be exchangeable below 100°.[11]

Experiments on the nonexchangeability of hemoglobin iron with ferric and ferrous ions are described in Chapter XI. Nor was the mag-

[3] G. Hevesy and A. H. W. Aten, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **14**, 5 (1939).

[4] C. Perrier and E. Segrè, *Ricerca sci.*, **9**, 628 (1938).

[5] A. H. W. Aten, *Dissertation*, Utrecht, 1939.

[6] L. Hahn and G. Hevesy, *Nature*, **145**, 549 (1940). H. J. Born, A. Lang, G. Schramm, and K. G. Zimmer, *Naturwissenschaften*, **29**, 222 (1941).

[7] D. E. Hull, *J. Am. Chem. Soc.*, **63**, 1269 (1941).

[8] J. N. Wilson, *J. Am. Chem. Soc.*, **60**, 2697 (1938).

[9] J. L. Tuck, *J. Chem. Soc.*, **1939**, 1292.

[10] E. Buch-Andersen, *Z. physik. Chem.*, **B32**, 237 (1936).

[11] H. H. Voge and W. E. F. Libby, *J. Am. Chem. Soc.*, **59**, 2474 (1937); H. H. Voge, *ibid.*, **61**, 1032 (1939).

nesium of chlorophylls a and b found to exchange with magnesium ions at room temperature in 80% acetone–water solution.[12]

Exchanges were found to take place between molecular iodine and diiodotyrosine and also between iodide and diiodotyrosine at pH 4.3. At pH 7.5, however, the reaction mixture was held at 37° C. for 2 days without significant exchange.[13] In 50% aqueous methanol solution at 50° interchange was found to take place between iodine and 3,5-diiodo-p-cresol, 2,6-diiodophenol, 4,6-diiodophenol, and 2,4,6-triiodophenol. The rate of reaction was not, as expected, that of a simple exchange process.[14] That no interchange takes place between radioactive iodine and nonradioactive iodate added in the course of the extraction of the iodine of tissues is of great importance for the application of the method of extraction applied in experiments with radioiodine.[15] In concentrated nitric acid solution an interchange was observed between iodate and iodine.[16] As noted in Chapter III, Armstrong and Schubert (personal communication) observed that barium carbonate samples kept in a moist atmosphere of carbon dioxide exchange their carbonate group with that of the atmosphere. ^{14}C was used as an indicator in these experiments; 25 mg. $BaCO_3$ per cm.^{2} in the course of two days lost 16% of its ^{14}C content while ^{14}C was found to be present in the atmosphere. Numerous results obtained in the study of exchangeability are discussed by Seaborg, by Ruben et al., and by Süe.[16a]

II. Exchange of Hydrogen

The considerations given above are not applicable in the case of hydrogen which has a unique position among the various elements. When ordinary sugar is dissolved in water containing deuterium oxide and the water is removed by distillation, the sugar remaining contains deuterium. If this sugar is dissolved in a large quantity of ordinary water and the water distilled off, the sugar loses its deuterium and passes

[12] S. Ruben, A. W. Frenkel, and M. D. Kamen, J. Phys. Chem., **46**, 710 (1942).

[13] W. H. Miller, G. W. Anderson, R. K. Madison, and D. J. Salley, Science, **100**, 340 (1944).

[14] H. A. C. McKay, J. Am. Chem. Soc., **65**, 702 (1943). P. Süe, Compt. rend., **212**, 237 (1941).

[15] I. Perlman, I. L. Chaikoff, and M. E. Morton, J. Biol. Chem., **139**, 433 (1941).

[16] M. Cottin and M. Haïssinsky, Compt. rend., **224**, 1636 (1947).

[16a] G. T. Seaborg, Chem. Revs., **27**, 256 (1940). S. Ruben, G. T. Seaborg, and J. W. Kennedy, J. Applied Phys., **12**, 308 (1941). P. Süe, J. Chim. Phys., **41**, 160 (1944).

over to the ordinary form.[17] This exchange between light and heavy hydrogen takes place with great rapidity. It is due to the attachment of deuterons from the water to the oxygen of the hydroxyl groups of the sugar and the splitting-off of protons by the latter in return. A large body of experimental data has shown that such a proton–deuterium exchange takes place in the case of all organic compounds containing hydroxyl or amine groups. For example, the investigation of the behavior of glycol,[18] hydrogen peroxide,[19] hydroquinone,[20] succinic acid, aniline,[21] and methylaniline[22] shows that two hydrogen atoms exchange speedily; one hydrogen atom, only, was found to exchange in the case of phenol,[23] benzyl alcohol,[21] benzoic acid,[21] acetanilide,[24] p-ethoxyacetanilide,[25] and dimethylamine.[22] Hydrogen atoms bound to oxygen and nitrogen are easily exchangeable with hydrogen atoms of water. In proteins in which about 14% of the hydrogen atoms are bound to oxygen or nitrogen, 14% of the hydrogen atoms were found to be exchangeable.[26] The replacement of hydrogen by deuterium is, in many cases, not due to a direct substitution but to the fact that ionization of the hydrogen of the compound in solution takes place and is followed by alternative recombination and dissociation. A detailed discussion of interchange reactions of deuterium with other molecules is given by Taylor.[27]

III. Removal of Radioactive Impurities

We make use of the lack of interchangeability of radioactive phosphate with phosphate of organic phosphorus compounds, for example, in the purification of labeled desoxyribonucleic acid from adhering labeled phosphate. When the latter is of much higher specific activity

[17] K. F. Bonhoeffer and G. W. Brown, *Z. physik. Chem.*, **B23**, 171 (1933).

[18] N. F. Hall, E. Bowden, and T. O. Jones, *J. Am. Chem. Soc.*, **56**, 750 (1934).

[19] H. Erlenmeyer and H. Gärtner, *Helv. Chim. Acta*, **17**, 970 (1934).

[20] A. E. Brodski and O. C. Scarre, *Acta Physicochim. URSS*, **2**, 603 (1935).

[21] M. Harada and T. Titani, *Bull. Chem. Soc. Japan*, **10**, 554 (1935); **11**, 465 (1936).

[22] P. Goldfinger and V. Lasarev, *Compt. rend.*, **200**, 1671 (1935).

[23] C. K. Ingold, C. G. Raisin, and C. L. Wilson, *J. Chem. Soc.*, **1936**, 1637.

[24] P. A. Small and J. H. Wolfenden, *J. Chem. Soc.*, **1936**, 1811.

[25] H. Erlenmeyer, A. Epprecht, H. Lobeck, and H. Gärtner, *Helv. Chim. Acta*, **19**, 354 (1936).

[26] H. H. Ussing, *Skand. Arch. Physiol.*, **78**, 225 (1938).

[27] H. S. Taylor, in R. E. Burk and O. Grummitt, *Advances in Nuclear Chemistry and Theoretical Organic Chemistry.* Interscience, New York, 1945.

its presence may entail a serious error.   By precipitating the nucleic acid repeatedly from a solution containing inactive phosphate we can successfully remove the adhering labeled phosphate ions.   By doing so we possibly replace the traces of active phosphate by traces of inactive phosphate; the presence of the latter will result in a slightly enhanced phosphorus content determined colorimetrically, but the error thus introduced will be far smaller than the error due to the presence of radioactive phosphate which might raise the activity figures of the phosphatide sample appreciably.   It is furthermore possible to remove the adhering nonactive phosphate by precipitation from phosphate-free solution in the later phases of the experiment.   The washing out of radioactive isotopes by an excess of nonactive isotopes is widely applied in investigations with radioactive tracers.

Loofbourow's survey on the application of physical methods to the investigation of biological and biochemical processes contains a list of papers published up to 1940,[28] while papers published up to 1942 are quoted in the review of Süe.[29]   Numerous papers published up to 1945 are discussed in *Les isotopes radioactifs en biologie* by Strohl and Berger.[30]

[28] J. R. Loofbourow, *Rev. Modern Phys.*, **12**, 267 (1940).

[29] P. Süe, *J. chim. phys.*, **40**, 31 (1943).

[30] A. Strohl and M. Berger, *Les isotopes radioactifs en biologie.*   Masson, Paris, 1946.

Application of
Isotopic Indicators in Chemical Analysis

I. Radioactive Control of Precipitate or Filtrate

Chemical analysis presents a vast field for the application of isotopic indicators. By making use of such tracers, we can check results of quantitative determinations of elements or compounds and correct for shortcomings of the methods employed. We wish to know, for example, whether the determination of the minute lead content of a sample is strictly quantitative. We then add to a solution of the sample a known amount of the lead isotope radium D of negligible weight. If the lead originally present and the radium D added are precipitated electrolytically on an anode as lead peroxide, and the activity measurement indicates the presence of only half the radium D added, we conclude that we have recovered only half the lead content of the sample. We must, therefore, multiply the amount of lead present in the lead peroxide precipitate by two to arrive at the lead content of the sample.[1]

In the determination of the rate of renewal of muscle creatine phosphoric acid (see page 255), the inorganic phosphate of the muscle extract is first precipitated as magnesium ammonium phosphate and its radioactivity is determined. The phosphate of creatine phosphoric acid is then split by acid hydrolysis and also precipitated as magnesium ammonium phosphate before determination of its radioactivity. The activity of 1 mg. inorganic phosphate is often higher than the activity of 1 mg. creatine phosphate. An incomplete precipitation of the inorganic phosphate will in such cases tend to give high values for the activity of 1 mg. creatine phosphate. We can account for this error by determining the amount of inorganic phosphate not precipitated as magnesium ammonium phosphate. Labeled phosphate of negligible weight (100.00 counter units) are added to one-tenth the muscle extract. If the filtrate of the magnesium ammonium phosphate precipitate has an activity of,

[1] G. Hevesy and R. Hobbie, *Nature*, **128**, 1038 (1931); *Z. anal. Chem.*, **88**, 1 (1932).

for example, 500 units, 5% of the free phosphorus of the muscle extract was not precipitated, and thus will be precipitated to a large extent later with the phosphate split off the creatine phosphoric acid.

When 2 ml. Mathison reagent and 5 ml. concentrated ammonia solution were added to 5 ml. of a solution containing a known amount of labeled phosphorus, the following amounts of phosphorus originally present were not precipitated[2]:

Free P originally present, mg.	P not precipitated, %
0.025	15
0.050	10
0.100	4

As an example of an isotopic check on quantitative precipitation we may cite the determination of zinc as sulfide. For verification we add labeled zinc of negligible weight to the solution containing the sample to be analyzed. By testing the radioactivity of the filtrate we can determine the percentage of zinc not precipitated as sulfide and we can easily correct for this error. The quantitative precipitation of almost every element can be checked by the use of an isotopic tracer.

As another example we may consider the radioactive checking of the result of a potassium determination using the method of Shohl and Bennet.[3] In this method, potassium is precipitated as potassium chloroplatinate. The solution is evaporated to dryness and washed with alcohol to remove excess chloroplatinate. The potassium chloroplatinate is converted by addition of potassium iodide to the wine-colored iodoplatinate. This is estimated colorimetrically or is titrated with thiosulfate. When labeled potassium of negligible weight was added to the solution to be analyzed, it was found that the alcohol used to rinse the potassium chloroplatinate precipitate contained an appreciable amount of labeled potassium. Using the amount of alcohol recommended[4] for the determination of 5 mg. potassium, 4% of the ^{42}K added, and thus 0.2 mg. potassium, was found in the alcoholic filtrate.[5] The efficiency of the Chaney method for the determination of plasma iodine has been tested with the aid of radioactive iodine.[6]

[2] K. Kjerulf-Jensen, *Acta Physiol. Scand.*, **3**, 9 (1941).

[3] A. T. Shohl and H. B. Bennett, *J. Biol. Chem.*, **78**, 643 (1928).

[4] B. Norberg, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, **21**, 233 (1938).

[5] G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **16**, 1 (1941).

[6] A. Taurog and I. L. Chaikoff, *J. Biol. Chem.*, **163**, 313 (1946).

In analyzing either serum or whole blood, by the dry ashing technique, radiosodium is occluded by the iron phosphate precipitate, the precipitated sodium not exceeding 0.5 to 1.2% of the total in the original solution.[6a]

II. The Method of Isotope Dilution

In biochemical analyses, where we are often faced with the determination of constituents of a mixture of numerous compounds of intricate constitution, isotopic tracers seem to be of great use. Rittenberg and Foster,[7] for example, determined the palmitic acid content of a mixture of fatty acids. They added deuteriopalmitic acid of known weight and known deuterium content to the mixture of isotopically normal fatty acids. They then recovered some palmitic acid and determined its deuterium content. If the sample contained much palmitic acid the fraction recovered would clearly have a low deuterium content and vice versa. From the amount of palmitic acid added (x), its deuterium content (C_0),[*] and the deuterium content of the isolated palmitic acid (C), the amount (y) of palmitic acid originally present in the mixture could be calculated from the equation:

$$y = \left(\frac{C_0}{C} - 1\right)x.$$

Since it is necessary, in this method of isotope dilution, merely to obtain a sample large enough for isotope analysis (5–100 mg.), large losses can be afforded in the purification process. In the determination of the palmitic acid of rat fat, of the original 3.5 g. palmitic acid actually present in each sample, only about 200 mg. was finally isolated and used for analysis. The accuracy of the method depends on three factors: the purity of the compound added, the purity of the compound isolated, and the accuracy of the isotopic analysis. With above 1 atom per cent excess for deuterium and 6.5 atom per cent excess for ^{15}N the error of a single isotope determination is about 1%. For the best results C should therefore exceed these values. The ratio C_0/C should also be as large as possible. Little advantage is gained, however, by increasing C_0/C above 10. It is advisable that the isotope concentration in the added

[6a] P. M. Hald, *J. Biol. Chem.*, **163**, 249 (1946).

[7] D. Rittenberg and G. L. Foster, *J. Biol. Chem.*, **133**, 737 (1940).

[*] All isotope concentrations are given as excess above normal. Normal hydrogen contains 0.02% deuterium, normal nitrogen 0.37% heavy nitrogen.

material be above 5 atom per cent. The results of the determination of palmitic acid in rat fat are seen in Table 17. The total fatty acids of 2 rat carcasses were obtained by the usual procedure, after hydrolysis of the fats in alcoholic potassium hydroxide for 6 hours. Two aliquots were taken, and deuteriopalmitic acid containing 21.5% deuterium was added.

TABLE 17

Determination of Palmitic Acid in Rat Fat[7]

Total fatty acids, g.	Palmitic acid added, g.	Deuterium content of sample isolated, %	Palmitic acid content calculated, %
14.641	0.2163	1.28	23.4
14.135	0.1757	1.18	22.9

Ussing[8] determined the leucine content of hemoglobin by the same method, making use of deuterioleucine as an indicator. In the determination of the leucine content of the rat,[9] leucine containing ^{15}N was employed; in determining glutamic acid, dl-glutamic acid containing heavy nitrogen was used.[10]

Kögl and Erxleben[11] had claimed that amino acids of the d-series, and particularly d(−)-glutamic acid, occurred as constituents in cancer proteins. This claim was disputed by other workers. Graff, Rittenberg, and Foster[10] showed by the isotope dilution method that malignant tissue contains l(−)-glutamic acid in amounts ranging from 6.7 to 8.7% of the total nitrogen. The other isomer d(−)-glutamic acid if present at all, could not have amounted to more than 1% of the total glutamic acid, i.e., not more than 0.067 to 0.087 of the total nitrogen. In this controversy, where the correctness of the analytical procedures was the chief debated point, the isotope dilution method proved to be of great value (see also Kögl et al.[12]).

The method was also used in the analysis of bovine and human serum albumin,[13] of hemoglobin, and of β-lactoglobulin.[14]

[8] H. H. Ussing, *Nature*, **144**, 977 (1939).

[9] R. Schoenheimer, S. Ratner, and D. Rittenberg, *J. Biol. Chem.*, **130**, 703 (1939).

[10] S. Graff, D. Rittenberg, and G. L. Foster, *J. Biol. Chem.*, **133**, 745 (1940).

[11] F. Kögl and H. Erxleben, *Z. physiol. Chem.*, **258**, 57 (1939).

[12] F. Kögl, H. Erxleben, and G. J. van Veersen, *Z. physiol. Chem.*, **277**, 251 (1943).

[13] D. Shemin, *J. Biol. Chem.*, **159**, 439 (1945).

[14] G. L. Foster, *J. Biol. Chem.*, **159**, 431 (1945).

The isotope dilution method was found useful in the search for bases other than ethanolamine and choline in organ phosphatides.[15] The pure bases, containing a known amount of the ^{15}N isotope, were added to the phosphatide hydrolysis mixture. These experiments showed that, whereas choline accounted for practically all the nonamino nitrogen in the phosphatide hydrolyzates, only 40–50% of the amino nitrogen was present as ethanolamine; the occurrence of at least one other primary base or hydroxyamino acid had therefore to be assumed. Also, in a mixture of ions, one of them can be determined by adding its radioactive isotope to the solution. The specific activity is modified, and one can deduce from the extent of the change the concentration of the inactive ion initially present. Potassium can, for example, be determined in the presence of lithium and sodium. To a solution containing these three elements, a measured quantity of labeled potassium having a known activity is added. From the new mixture the potassium is precipitated as the perchlorate, isolated, and weighed; and from the new activity the amount of inactive potassium ions initially present in the solution is calculated.[16]

The three individual components of mixtures of dibenzyl sulfide, sulfoxide, and sulfone[17] have been determined by the isotope dilution technique.

Within the last year, Keston, Udenfriend, and Cannan[18] have devised a very sensitive modification of this analytical procedure, and described its application to the analysis of amino acids in a mixture. In the modified method, a labeled substance that will form a stable derivative of the compound to be analyzed is added first. The isotope content is Cr. A great excess (W) of unlabeled derivative is then added, and the derivative is purified to constant isotope concentration Cc. The amount of derivative initially present is then equal to $W(Cc/Cr)$. An exact set of equations for isotopic dilution experiments has been devised by Gest and co-workers.[19]

[15] E. Chargaff, M. Ziff, and D. Rittenberg, *J. Biol. Chem.* **138**, 439 (1941); **144**, 343 (1942).

[16] P. Süe, *Nature,* **157**, 622 (1946).

[17] F. C. Henriques, Jr., and C. Margnetti, *Ind. Eng. Chem., Anal. Ed.*, **18**, 476 (1946).

[18] A. S. Keston, S. Udenfried, and R. K. Cannan, *J. Am. Chem. Soc.*, **68**, 1390 (1946).

[19] H. Gest, M. D. Kamen, and J. R. Reiner, *Arch. Biochem.*, **12**, 273 (1947).

CHAPTER VI

Absorption, Distribution, and Excretion of Elements

I. General Remarks

By labeling the atoms present in the food we can trace their path through the body, determine the time they spend in the various compounds and organs in which they are incorporated, and follow the path through which they ultimately leave the organism.

II. Absorption from Intestine

With the aim of comparing the rate at which sodium, potassium, chlorine, bromine, and iodine ions are absorbed from the alimentary tract, Hamilton[1] administered labeled salts of these ions to fasting human subjects. In the case of radiosodium, radiopotassium, and radiobromine, 100 ml. isotonic solutions were used; smaller amounts of radiochlorine and radioiodine were employed.

The γ-radiation emitted by the hand was used as an indicator of absorption. The subject held in his hand a small, glass-walled counting tube. This tube was encased in a copper tube with walls 1 mm. thick to screen out all β-rays. A large lead box with walls 12.5 cm. thick and open at one end had been constructed; in this the counting tube was held by the patient. This arrangement shielded the counting tube from the γ-radiation emitted from other parts of the patient's body.

The results obtained are seen in Figure 14. The rates of absorption of sodium, chlorine, bromine, and iodine were found to be rather rapid, the radioactive atoms of each of the four substances being detected in the hand within 3 to 6 minutes after ingestion. Absorption, as far as it took place, was completed within 3 hours. Potassium was absorbed more slowly, the labeled atoms not being detected earlier than 6 to 15 minutes after ingestion of the salt, and absorption was not completed before 5 hours had elapsed. The figures obtained do not

[1] J. G. Hamilton, *Am. J. Physiol.*, **124**, 667 (1938).

indicate what percentage of the administered element was absorbed, but they show the time after which no further increase in content of absorbed element occurs in the hand and moreover, the rate at which this maximum is reached. If we wish to know the percentage of an
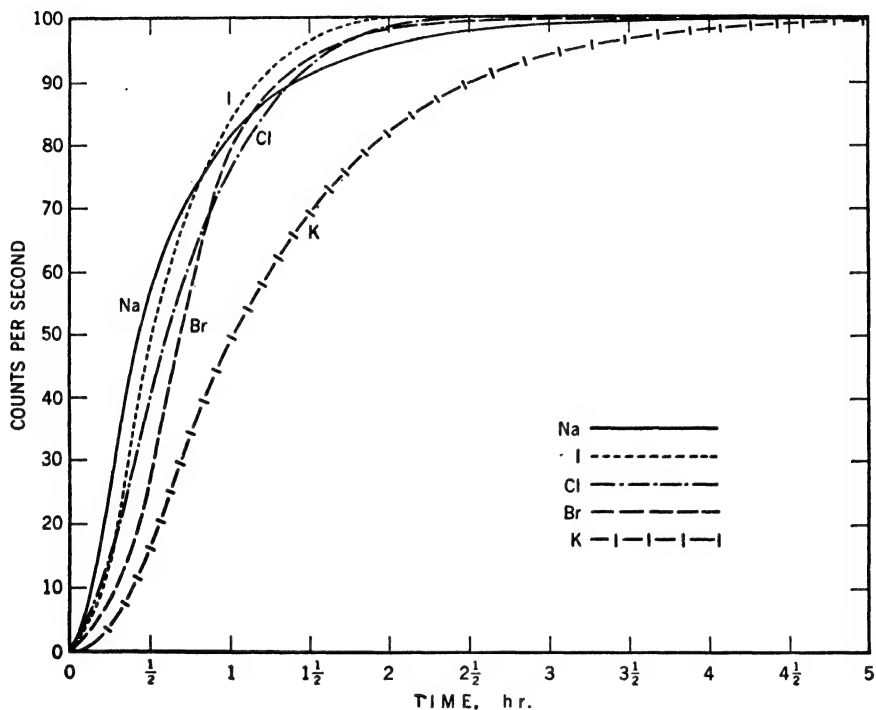
Fig. 14.   Absorption of ions from the alimentary tract of fasting human subjects.[1]

element administered which is absorbed during a given time, we must determine what percentage of the radioactivity administered is still present after the lapse of that time in the digestive tract or, as an alternative, we must determine the activity of the total body exclusive of the digestive tract. In such experiments due regard must also be paid to the amount excreted. In both cases, we must furthermore envisage the possibility that part of the activity present in the digestive tract is endogenous in origin, that is, the part that was actually absorbed, but reached the digestive tract again through the digestive juices.[2] In experiments of short duration, the fraction of endogenous activity in

[2] G. Hevesy, *Enzymologia*, **5**, 148 (1938).

the total activity of the digestive tract can often be disregarded (see page 103).

III. Phosphorus

A. ABSORPTION AND EXCRETION

By measuring the activity of known aliquot parts of the urine and the feces excreted, the daily loss of ^{32}P through both the kidneys and the bowels can be followed. Numerous such determinations were carried out,[2-6a, 9] the values found showing great variation for different individuals, much depending also on the diet given. Five milligrams labeled phosphorus as orthophosphate, pyrophosphate, yellow phosphorus, and red phosphorus, respectively, were given to fasting rats weighing about 200 g.[2] The amount of labeled phosphorus absorbed within 4 hours was found to be 89.3, 84.8, 80.2, and 35 0% respectively. In another set of experiments,[3] the gross absorption of the ^{32}P administered as orthophosphate (1.3 mg.) to fasting rats was found after 1, 2, 8, and 8.5 hours, to be 44, 54, 96, and 99%, respectively. From the 72.4 mg. phosphorus administered daily to rats weighing 234 g. and kept on normal diet, 71.5% was found to be absorbed.[7] In experiments in which 4.5 to 13 mg. phosphorus as labeled sodium phosphate was administered to rats weighing 220–300 g., the major portion of absorption was found to occur within the first 2 hours and the stomach was usually found to be empty within 4 hours. Radiophosphorus was observed to be present in the large intestine and the cecum within 2 hours. After the lapse of 8 hours, in most cases the small intestine was already free[5] of [32]P. Chicks retained 77% of the ^{32}P administered in the sixty-day period.[2a]

[2a] S. F. Scott, K. G. Scott, and P. Abelson, *Proc. Natl. Acad. Sci. U. S.*, **23**, 528 (1937).

[3] M. J. L. Dols, B. C. P. Jansen, G. Sizoo, and J. de Vries, *Koninkl. Akad. Wetensch. Amsterdam*, **40**, 547 (1937).

[4] W. E. Gaunt, H. D. Griffith, and J. T. Irving, *J. Physiol.*, **100**, 372 (1941/42).

[5] W. E. Cohn and D. M. Greenberg, *J. Biol. Chem.*, **123**, 185 (1938).

[6] O. Chievitz and G. Hevesy, *Nature*, **136**, 754 (1935); *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **13**, 9 (1937). L. A. Erf, L. W. Tuttle, and J. H. Lawrence, *Ann. Internal Med.*, **15**, 487 (1941). L. Hahn, G. Hevesy, and E. Lundsgaard, *Biochem. J.*, **31**, 1705 (1938). L. Hahn, G. Hevesy, and O. Rebbe, *Biochem., J.* **33**, 1549 (1939). J. H. Lawrence, K. G. Scott, and L. W. Tuttle, *New Intern. Clinics*, III, 33 (1939). L. A. Erf and J. H. Lawrence, *Ann. Internal Med.*, **15**, 276 (1941).

[6a] F. T. Hunter and A. F. Kip, *J. Applied Phys.*, **12**, 324 (1941).

[7] K. Kjerulf-Jensen, *Acta Physiol. Scand.*, **8**, 1 (1941).

The percentage of ^{32}P (administered subcutaneously as 0.1 ml. labeled sodium phosphate, pH 7.6) which was excreted in the course of the first 24 hours by fasting rats is seen in Table 18. Data are also given for rats which were irradiated with a Röntgen ray-dose of 1000 r. before administration of ^{32}P. The figures represent the averages obtained in the investigation with several rats weighing 140–160 g. In other experiments, in the course of the first 90 hours, 24% of the ^{32}P administered was found to be lost through the kidneys and 2.6% through the bowels.[4]

TABLE 18

Excretion of ^{32}P by Rats in First Twenty-Four Hours
Following Subcutaneous ^{32}P Administration[8]

Mode of excretion	Excreted ^{32}P, per cent of administered	Per cent ^{32}P administered per milligram excreted phosphorus
Controls		
Urine	8.5	0.94
Feces	0.23	0.24
Irradiated		
Urine	10.9	1.41
Feces	0.62	0.19

Experiments were carried out on mice with the aim of determining whether the whole body retention of orally administered radiophosphorus could be increased or decreased by simultaneous administration of various foods or salts.[9] After feeding 3.7 mg. labeled sodium phosphate to mice, the retention figures shown in Table 19 were obtained.

TABLE 19

Retention of Phosphorus[9]

Agent used	Average retention of ^{32}P after 3 days, per cent per gram
Water	1.26
Regular diet	1.30
Glucose	1.71
Ferrous sulfate	0.82

[8] G. Hevesy, *unpublished data.*

[9] L. A. Erf, L. W. Tuttle, and K. G. Scott, *Proc. Soc. Exptl. Biol. Med.*, **45,** 652 (1940).

Glucose thus increased and iron salts decreased the absorption of radioactive phosphorus from the gastrointestinal tract. This result is presumably due to an enhanced absorption from the intestine in the presence of glucose and to a decreased absorption owing to the excretion of iron phosphate through the bowels. The average ^{32}P retention by mice on a regular diet is 30% of the amount administered. A 125-fold increase in amount of phosphorus fed (from 0.06 to 7.5 mg. Na_2HPO_4) causes a decrease in percentage retention to almost half its value.

In experiments in which fasting rats were killed 10 minutes after ingestion of labeled phosphate, the absorbed amount of ^{32}P in stomach, duodenum, jejunum, and ileum did not differ more than 30% when glucose was administered simultaneously.[10]

The excretion of radiophosphorus in the urine of rats maintained on inadequate dietary phosphorus is only one-fourth that found in the urine of rats on a normal diet, the corresponding ratio for the radiophosphorus content of the feces being 3 : 4. The small difference in radiophosphorus content of the feces indicates that the phosphorus secreted in the digestive tract does not differ much in rats kept on diets inadequate or adequate in phosphorus.[4]

Vitamin D was found to increase the rate of renal tubular phosphate absorption, thereby increasing the concentration of this ion in the plasma and other extracellular fluids. On the other hand, parathyroid extract decreases the rate of reabsorption of phosphate by the renal tubules and lowers the concentration of phosphate in the plasma.[11] The absorption of phosphate by rachitic rats is increased to a minor extent by vitamin D.[5,12] The decrease of fecal phosphorus from 31 to 20% in rachitic rats caused by vitamin D is due to both increased absorption from and decreased re-excretion into the gut. Urinary phosphorus excretion in the first 64 to 80 hours amounts to only 0.16% of the dose administered by stomach tube and to 0.9% of the dose given by intraperitoneal injection. No change in the percentage excretion could be found after administration of vitamin D in these experiments.[5] Data are available on the uptake of ^{32}P by rachitic chicks.[13]

The amount of ^{32}P in the circulation is the difference between the

[10] J. H. Perryman, R. de la Madrid, and S. C. Brooks, *Science*, **100**, 271 (1944).

[11] H. E. Harrison and H. C. Harrison, *Am. J. Physiol.*, **134**, 781 (1941).

[12] K. Morgareidge and M. Manly, *J. Nutrition*, **18**, 411 (1939).

[13] M. J. Dols, B. C. P. Jansen, G. J. Sizoo, and F. Barendregt, *Koninkl. Akad. Wetensch. Amsterdam*, **41**, 997 (1938).

amount absorbed into the circulation and the amount which either passed from the circulation into the organs or was excreted. The maximum ^{32}P content of the plasma of the rat was observed to be reached 14 minutes after intraperitoneal injection of labeled phosphate,[7] while this state was attained in the plasma of the rabbit 20–30 minutes after subcutaneous injection[14] (see Figure 15), the time being dependent on the injected volume. The rapid decrease in the ^{32}P content of the plasma after intravenous injection is illustrated in Figure 16. Intraperitoneally injected ^{32}P in mice showed maximum activity in the blood after the lapse of 11 hours.[14b]
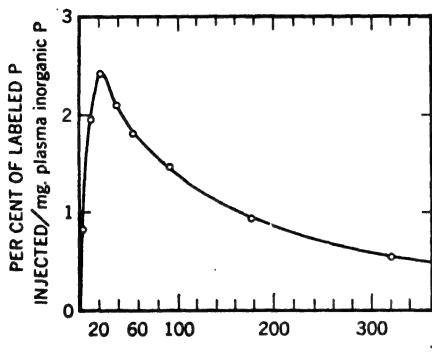
Fig. 15. Change in specific activity of plasma inorganic phosphorus of a rabbit after subcutaneous injection of labeled phosphate.[14]
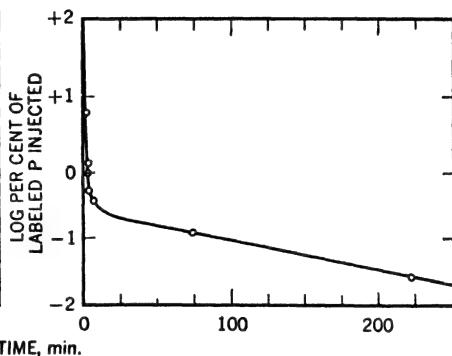
Fig. 16. Logarithmic change in specific activity of plasma inorganic phosphorus of a rabbit after intravenous injection of labeled phosphate.[14]

In a study of the rate of disappearance of ^{32}P from the blood of *Limulus* and *Homarus*, labeled phosphate was injected into the hearts of the animals. At the end of 1 hour, 97.6% of the ^{32}P administered had disappeared from the circulation of *Limulus polyphemus*. The rate of disappearance was much lower for *Homarus americanus*.[14a]

When ^{32}P is introduced into the duodenum of the dog, labeled phosphorus will appear in the general circulation within 5 minutes. The maximum change in ^{32}P content of the portal and jugular blood occurs within the first half-hour; the portal ^{32}P level exceeds that of jugular

14 G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **15**, 5 (1940).

14a W. H. Cole and W. L. Nastuk, *Proc. Soc. Exptl. Biol. Med.*, **48**, 151 (1941).

14b H. B. Jones, *personal communication*.

and carotid blood significantly for 3 to 3.5 hours. The maximum specific activity of the total liver phosphorus is reached after about 2.5 hours. At the end of 4 hours, 0.4 to 5% of the dose can be washed out of the small intestine, while the rest disappears from the gut. The intestinal tissue was found to contain 20% of the dose, of which 90% was located in the mucosa; 15% of the dose was found in the liver, 9% in the blood, 13% in the muscle, and 10% in the skeleton.[15] When labeled phosphorus was administered to rats as aminoethylphosphoric acid 28% was excreted through the kidneys in the course of 8 hours.[15a]

B. EXCRETION BY HUMAN SUBJECTS

The rates of excretion of ^{32}P administered together with significant amounts of Na_2HPO_4 orally or by intravenous injection to human subjects, determined by Erf, Tuttle, and Lawrence,[16] are shown in Table 20 (see also Hevesy et al.[17] and Lawrence and co-workers[18]). Normal individuals were found to excrete ^{32}P more rapidly than patients with leukemia during the first 48 hours after administration. The rates after the 48-hour period are similar. The amount of labeled phosphate excreted by human subjects within the first 24 hours through the kidneys was found to vary between 4.0 and 23.0% of the dose injected

TABLE 20

Rate of Excretion of ^{32}P in Urine and Feces[16]

Excreted in	Per cent excreted						Total excreted
	Days after ^{32}P administration of 600 mg. Na_2HPO_4 containing 1500 μc. ^{32}P						
	1	2	3	4	5	6	
Oral administration							
Urine	4.63	1.7	0.89	0.72	0.74	0.45	22.9
Feces	12.93	0.52	0.22	0.02		0.06	
Intravenous administration							
Urine	37.66	5.03	2.48	1.69	1.74	1.16	50.05
Feces	0.098	0.25	0.24	0.16	0.02	0.05	

[15] L. H. Weissberger and E. S. Nasset, Am. J. Physiol., 138, 149 (1942/43).

[15a] E. Chargaff and A. S. Keston, J. Biol. Chem., 134, 515 (1940).

[16] L. A. Erf, L. W. Tuttle, and J. H. Lawrence, Ann. Internal Med., 15, 487 (1941).

[17] G. Hevesy, L. Hahn, and O. Rebbe, Kgl. Danske Videnskab. Selskab Biol. Medd., 14, 3 (1939).

[18] J. H. Lawrence, K. G. Scott, and L. W. Tuttle, New Intern. Clinics, III, 33 (1939). L. A. Erf and J. H. Lawrence, J. Clin. Invest., 20, 567 (1941).

intravenously. The retention of ^{32}P by patients was practically identical, regardless of whether magnesium ammonium phosphate, phosphoric acid, or sodium phosphate was administered intravenously.[19] In experiments taking 43 days, ^{32}P values per milligram urine P ranging from 0.015 to 0.00016% of the ^{32}P administered have been reported. Govaerts and Lambrechts[20] found that 2.53% of the ^{32}P injected intravenously into humans was eliminated by the urine in the first 4 hours, the corresponding figures being 0.96 and 1.13 after 24 hours and 4 days, respectively (*cf.* also Govaerts[22]).

The retention of ^{32}P when administered intravenously to leukemic patients is relatively constant, amounting on the third day to 75% of the original dose and on the seventh day to 52%. The rate of excretion of radiophosphorus by leukemic patients was furthermore found by Warren[21] not to vary significantly with variation of doses within the therapeutic range nor with the clinical condition of the patient. The feces collected for 6 days contained only a few thousandths of the dose administered. Although a slight amount of ^{32}P is excreted in the saliva, this is usually swallowed, and then the major part is reabsorbed. In one case, the saliva contained 0.042 microcurie per milliliter 1.5 hours after injection of a dose of 2606 microcuries, when the blood level was 0.04 microcurie per milliliter[21] (see also Warren[21a]). One hour after ingestion of 4 g. labeled sodium phosphate by a human subject, the specific activity of the saliva phosphorus was found to be 16% of that of plasma phosphorus.[21b]

During the first minutes following injection of massive doses of labeled phosphate into the veins of the dog, the specific activity of the acid-soluble urine phosphorus was appreciably higher than the specific activity of the acid-soluble plasma phosphorus. The difference between the specific activities diminishes with time and disappears entirely after the lapse of 1.5 hours. Govaerts[22] concludes from this observation that inorganic phosphorus comprises only a minor part of the total acid-soluble phosphorus of plasma. The change in ^{32}P concentration of the blood following administration of ^{32}P is discussed on page 100 and in Chapter XI.

[19] S. Warren and R. F. Cowing, *Cancer Research*, **4**, 113 (1944).

[20] J. Govaerts and A. Lambrechts, *Bull. soc. roy. sci. Liége*, **11**, No. 2, 138 (1942).

[21] S. Warren, *New Eng. J. Med.* **223**, 751 (1940).

[21a] S. Warren, *Cancer Research*, **3**, 872 (1943).

[21b] C. P. Barnum and W. D. Armstrong, *Proc. Soc. Exptl. Biol. Med.*, **49**, 40 (1942).

[22] J. Govaerts, *Bull. acad. roy. méd. Belg.*, **9**, 624 (1943); *Nature*, **160**, 53 (1947).

C. ORIGIN OF FECES PHOSPHORUS

The balance of phosphorus intake and excretion has been investigated in numerous cases. A vast literature on this subject is available in which the route of secretion is often also considered, *i.e.*, the ratio of urinary to fecal phosphorus of the human subject or the animal investigated.[23]

The percentage of phosphorus excreted in the feces due to unabsorbed material and the amount due to phosphorus originating from the body proper were, however, not known before the application of isotopic indicators. By using radiophosphorus as an indicator, Hevesy *et al.*[17] determined to what extent fecal phosphorus is composed of unabsorbed (exogenous) phosphorus and to what extent of phosphorus derived from the body proper.

Let us assume that all phosphorus present in the food is absorbed into the circulation. Then all labeled phosphorus found in the feces must originate from the body proper. It is ultimately the plasma inorganic phosphorus that is responsible for the formation of the phosphorus compounds present in the digestive juices; and, therefore, the specific activity (activity per milligram of phosphorus) of the feces phosphorus should, in the case mentioned above, be equal to that of plasma phosphorus. The specific activity of the inorganic plasma phosphorus being equal to that of the urine phosphorus, we shall expect to find the specific activity of the feces phosphorus equal to that of the urine phosphorus. If the above assumption does not hold and if part of the feces phosphorus consists of unabsorbed, inactive phosphorus originating from the undigested food, the specific activity of the feces phosphorus will be found to be lower than that of the urine phosphorus. The ratio:

$$\frac{\text{specific activity of feces phosphorus}}{\text{specific activity of urine phosphorus}} \times 100$$

gives the per cent phosphorus of the feces which originates from the body proper. If food phosphorus is, for example, quantitatively absorbed, the above ratio will be 100. The objection that might be raised against the above considerations is that the digestive juices contain not only acid-soluble phosphorus compounds, most of which become labeled within a short time, their activity per milligram being equal to that of the specific activity of the plasma inorganic phosphorus, but also phosphatides, and other phosphorus compounds which are renewed and thus labeled (see Ch. VIII) at a slow rate. This objection

[23] R. Nicolaysen, *Biochem. J.*, **31**, 107 (1937).

will not be valid, however, if we start to collect the urine and feces samples some time after the administration of the labeled phosphorus. After the lapse of a considerable time, most of the phosphorus present in the different compounds of the organs responsible for the production of the digestive juices will be in exchange equilibrium with the plasma phosphorus, and will thus have about the same specific activity.

Twenty-eight days after subcutaneous injection of labeled sodium phosphate into a female subject the specific activity of urine phosphorus was found to be 8.08, and for fecal phosphorus the value was 1.77. Of the total phosphorus of the feces, 18% was residual phosphorus obtained after removal of acid-soluble phosphorus (mostly calcium phosphate) and traces of phosphatide phosphorus. The specific activities of the different phosphorus fractions varied only to a minor extent. The specific activity of the feces phosphorus is only 22% of the specific activity of the urine phosphorus; the feces phosphorus, therefore, to a large extent must have originated from unabsorbed food, which is the only source of nonactive phosphorus. From the above figures it follows that 78% of the phosphorus present in the feces of the human subject in question was unabsorbed phosphorus; the rest originated from the body proper. This result is, however, not to be interpreted as an indication that only 22% of the food phosphorus was absorbed. When interpreting the above figures we must take into account that the phosphorus excreted through the kidneys constitutes about twice that lost through the bowels; the sum of both values represents the total phosphorus present in the food, if we assume that the subject is in phosphorus balance. Then we find that only 26% of the total phosphorus of the food was not absorbed into the circulation.

A detailed investigation of the excretion of phosphorus by the bowels was carried out by Kjerulf-Jensen.[24] In order to test the supposition that the specific activity of urinary phosphorus parallels that of digestive secretions, he compared the specific activity of fecal phosphorus from rats kept on a phosphorus free diet. He was able to support the above supposition.

The same investigator compared the specific activity of the total phosphorus in samples of bile–pancreatic juice with the specific activity of urinary phosphorus and found that one week after administration of labeled phosphorus the specific activity of the total phosphorus in bile–

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[24] K. Kjerulf-Jensen, *Acta Physiol. Scand.*, **3**, 1 (1941).

pancreatic juice samples was already very near the value of urinary phosphorus. He obtained values for the ratio:

$$\frac{\text{specific activity of bile phosphorus}}{\text{specific activity of urine phosphorus}} \times 100$$

of 84, 98, 96, and 99 at 6, 6, 11, and 31 days, respectively, following ^{32}P administration. It may therefore be considered justifiable to regard the specific activity of the urine as an indicator of that fraction of the fecal endogenous phosphorus which originates in the digestive secretions.

TABLE 21

Endogenous Fecal Phosphorus of Human Subjects[24]

Sampling period, days after ^{32}P given	Average total urine P per 24 hr., mg.	Average total fecal P per 24 hr., mg.	Average endogenous fecal P in per cent of total fecal P	Average endogenous fecal P produced in 24 hr., mg.
5–11	980	457	24.8	105
6– 9	820	617	25.0	138
8–18	305	370	27.5	93
30–34	1050	338	31.0	102

TABLE 22

Phosphorus Balance Study during Six-Day Period
of Mature Rats on Normal Diet[24]

Condition	Milligrams P per 100 g. rat per 24 hr. (av. values for 5 rats)
Given by mouth	31.0
Excreted in urine	7.4
Excreted in feces	15.3
Retained	8.3
Endogenous fecal P, per cent of total fecal P	43.5
Absorbed P, per cent of administered P	71.5

To determine the amount of endogenous fecal phosphorus, four adult persons consuming an ordinary mixed diet were studied. The results obtained are shown in Table 21. For rats on a normal diet, values for average phosphorus balance are shown in Table 22.

When interpreting the figures for endogenous and exogenous phosphorus content in the feces, we must envisage the possibility that some phosphate interchange takes place through the intestinal wall and that, correspondingly, the endogenous phosphorus present in-the feces may be partly secreted and partly interchanged phosphorus. Indications of such an interchange were observed by Kjerulf-Jensen.

Data on the amount of endogenous phosphorus present in the feces were also obtained by chemical determination of the phosphorus content of the feces of fasting animals.[23] The conditions in these experiments were, however, far from physiological. The amount of digestive juices and, thus, the amount of phosphorus secreted into the digestive tract depend largely upon the amount and quality of the food administered. When, for example, 50 g. oil and 300 g. labeled phosphorus (as sodium phosphate) were administered to a fasting dog,[25] the total phosphorus of the intestinal tract, after five hours, contained about 75% phosphorus of endogenous origin.

Govaerts[26] et al., found the endogenous part of fecal phosphorus to be increased after administration of vitamin D to human subjects. Furthermore, the % excretion of ^{32}P both through the kidneys and the bowels was found to be increased after administration of this vitamin.

The method outlined above can be used to determine what percentage of almost any element present in the feces is of endogenous origin. Of the sodium output of human subjects, 1.2 to 5.1% and, of the potassium output, 6 to 13% were found to be present in the feces.[26a] It is not yet known which part of the fecal sodium and potassium is unabsorbed material and what part originates from the body proper.

D. DISTRIBUTION OF RADIOPHOSPHORUS

1. Distribution in Organs of the Rat

In the early phases of distribution studies, about the same percentage of the administered labeled phosphorus (mostly Na_2HPO_4) is found in the muscles and in the skeleton (see Table 23). With increasing time more labeled phosphate accumulates in the skeleton (see Table 24), as discussed in greater detail in Chapter X.

Cohn and Greenberg[27] found (Figure 17) that retentions per unit

[25] G. Hevesy and E. Lundsgaard, *Nature*, **140**, 275 (1937).
[26] J. Govaerts and A. Lambrechts, *Bull. soc. roy. sci. Liége*, **11**, No. 3, 361 (1942).
[26a] G. Hevesy, *Acta Physiol. Scand.*, **3**, 123 (1942).
[27] W. E. Cohn and D. M. Greenberg, *J. Biol. Chem.*, **123**, 185 (1938).

fresh weight of tissue decreased in the following order: bones, liver, stomach plus small intestine, heart, kidneys, lungs, blood, muscles, skin, and brain (see also Born[29]). The distribution of the ^{32}P retained among the different phosphorus compounds present in the tissue of the organs is discussed in Chapter VIII.

TABLE 23

Distribution of ^{32}P between Different Organs of a Rat Four Hours
after Subcutaneous Injection of Labeled Phosphate[28]

Organ	^{32}P present, %	Specific activity
Bones.....................	22.6	0.020
Muscles..................	18.7	0.191
Liver.....................	17.6	0.475
Digestive tract............	15.9	0.365
Skin.....................	11.1	0.192
Lungs and heart...........	6.3	0.317
Blood....................	2.5	0.558
Kidneys..................	2.4	0.370
Spleen....................	1.3	0.256
Brain....................	0.02	0.032

TABLE 24

Percentage Distribution of ^{32}P in Various Organs of the Rat[28]

Organ	Time after administration of labeled phosphate						
	Hours		Days				
	0.5	4	10	20	30	50	98
Muscles..........	18.3	19.4	25.8	28.8	25.2	12.1	3.6
Skeleton..........	19.1	23.4	43.1	43.1	51.8	76.5	92.0

Gaunt and associates[30] report the following data (Table 25) for the percentage distribution of radiophosphorus 90 hours after subcutaneous injection (averages for 4 rats).

When the rats were fed from weaning a diet poor in calcium and phosphorus, the skeleton, 90 hours after administration, contained only 38.3% of the radiophosphorus fed. Thus, the skeleton of the rats on the better diet retained a higher percentage of ^{32}P than did the skeleton of the rats on the poorer diet. The muscles of the latter group took

[28] G. Hevesy, *J. Chem. Soc.*, **1939**, 1213.
[29] H. J. Born, *Naturwissenschaften*, **28**, 476 (1940).
[30] W. E. Gaunt, H. D. Griffith, and J. T. Irving, *J. Physiol.*, **100**, 372 (1941/42).

up 36.4%, thus more than was taken up by the muscles of rats fed adequate calcium and phosphorus.
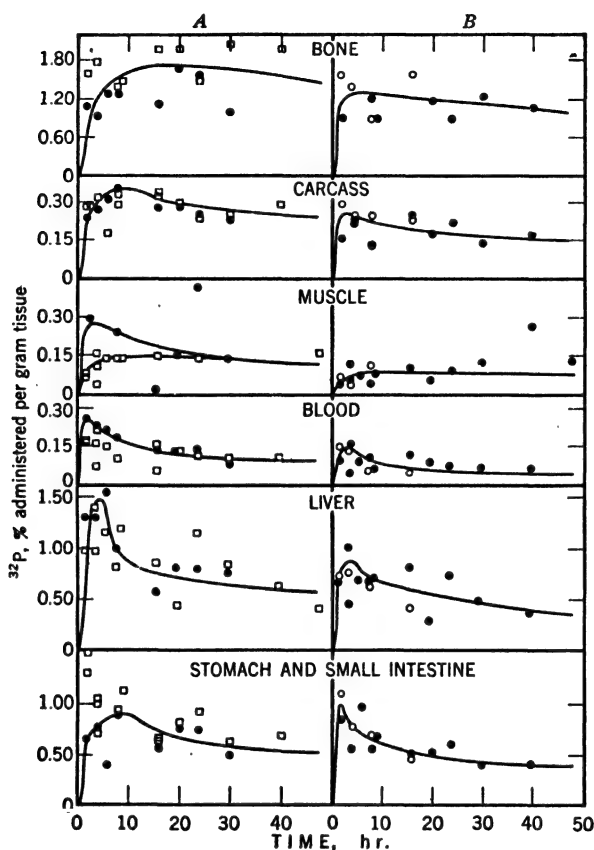
Fig. 17. Retention of radiophosphorus in various tissues and in blood per gram fresh weight.[27] *A*, administered by intraperitoneal injection: ● Series II, □ Series I and III (stomach tube) in terms of per cent absorbed radiophosphorus. *B*, administered by stomach tube: ● Series I (6 to 13 mg. P), ○ Series III (4.5 mg. P).

TABLE 25

^{32}P Distribution in the Rat Ninety Hours after Administration[30]

Organ	^{32}P, %	Organ	^{32}P, %
Skeleton	48.30	Muscles	27.38
Incisors	2.14	Liver	4.30
Molars	0.77	Viscera	4.30
Brain	0.48	Kidneys	8.86
Skin	4.63		

Parathyroid extracts were found by Tweedy and Campbell[31] to promote accumulation of labeled phosphate in the liver and the kidneys of rats (Table 26). The extracts produced an immediate increase in urinary excretion of radiophosphorus and a decrease in fecal excretion that was first discernible 18 to 24 hours after administration of labeled phosphate.

With increasing age, the specific activity of the organ phosphorus decreases. This is due partly to an increased phosphorus content and partly to a decreased turnover of phosphorus in the aging organism. When the age increases from six to twenty-four weeks, the specific activity of tibia phosphorus decreases from 0.94 to 0.08, that of spleen phosphorus from 2.78 to 0.96, and that of liver phosphorus from 3.1 to 1.6. Thyroxine was found to affect the specific activity of tibia phosphorus only, increasing the value from 0.08 to 0.11.[32] In no tissue does excess vitamin E cause an increase in ^{32}P uptake at all age levels, nor does it cause a significant increase in ^{32}P uptake of all tissues at any age level.[33]

Lindberg[34] compared the uptake of labeled propanediol phosphate by rat liver with the uptake of labeled orthophosphate of the same activity. While the maximum uptake was reached in about 1 hour after intraperitoneal injection of the orthophosphate, the maximum uptake of propanediol was observed after the lapse of only about 15 minutes. This result supplies another example of the rapid uptake by the liver of phosphorus compounds which are not normally present in the circulation. In the above experiments, after acid hydrolysis of the trichloroacetic-acid-soluble phosphate fractions of the liver, the specific activities of the fractions obtained after 10- and 20-minute hydrolyses were higher after injecting propanediol phosphate than after the injection of orthophosphate of equal activity.

2. Distribution in Organs of the Mouse

For the distribution of ^{32}P in the organs of the mouse 24 hours after intravenous administration, the following figures were found by Pecher[35] (see Table 27).

[31] W. R. Tweedy and W. W. Campbell, *J. Biol. Chem.*, **154**, 339 (1944).

[32] M. Falkenheim, *Am. J. Physiol.*, **138**, 175 (1942).

[33] L. H. Weissberger and P. L. Harris, *J. Biol. Chem.*, **151**, 543 (1943).

[34] O. Lindberg, *Svenska Vetenskapsakad. Arkiv Kemi*, **A23**, No. 2 (1946).

[35] C. Pecher, *Proc. Soc. Exptl. Biol. Med.*, **46**, 86 (1941).

TABLE 26

Effect of Parathyroid Extract upon Distribution, Retention, and Excretion[a] of Labeled Phosphorus[31]

Rat No.[b]	Weight, grams	Time after injection of 32P, hr.	Blood, per g.	Liver		Muscle, per gram	Femur		Kidney		Stomach, small intestine, and contents (whole)	Large intestine and contents (whole)
				per whole organ	per gram		per whole bone	per gram	per whole organ	per gram		
E-1	204	1	0.19	16.89	1.42	0.12	3.11	2.55	2.19	1.16	6.82	2.32
C-1	204	1	0.25	13.15	1.41	0.13	3.39	2.92	2.19	1.10	5.60	2.21
E-2	202	1	0.22	14.79	1.80	0.11	3.38	2.80	2.65	1.49	6.43	2.32
C-2	204	1	0.24	13.54	1.41	0.14	3.45	2.90	2.09	1.11	6.43	2.31
E-3	196	2	0.22	10.67	1.77	0.17	1.16	1.08	2.04	1.32	3.11	2.91
C-3	196	2	0.19	8.72	1.34	0.19	1.03	0.87	1.70	1.06	3.27	2.92
E-4	183	2	0.17	9.03	1.40	0.14	1.17	1.07	1.85	1.18	4.48	1.31
C-4	175	2	0.19	8.41	1.01	0.18	0.97	0.85	1.45	0.97	4.47	1.48
E-5	247	2	0.17	13.14	1.56	0.18	1.04	0.75	2.03	0.99	4.57	1.66
C-5	255	2	0.16	9.89	1.29	0.08	0.97	0.69	1.94	0.93	4.74	1.90

a Values for all tissues are expressed as per cent of administered radiophosphorus recovered.
b E represents experimental, C control.

The maximum deposit of ^{32}P, as was found by Jones and co-workers,[36] is reached between 5 and 10 hours after the start of the experiment. Of the soft tissues the highest activity was found in liver, small

TABLE 27

Distribution per Gram Wet Weight of ^{32}P in Organs of the
Mouse Twenty-Four Hours after Intravenous Injection[35]

Organ	^{32}P, %/g.	Organ	^{32}P, %/g.
Bone	5.2	Digestive tract	1.3
Muscle	1.4	Liver	3.0
Skin and hair	0.75	Other viscera	2.1

intestine, and kidney; next in order were cardiac and skeletal muscles, lymph nodes, and lungs. The lowest activity was exhibited by blood and brain (Table 28).

TABLE 28

Maximum Uptake of ^{32}P by Organs of Mice[36] Weighing 20 to 25 Grams

Organ	Maximum uptake/g. fresh tissue, %	Organ	Maximum uptake/g. fresh tissue, %
Liver	9	Muscle	3.9
Small intestine	6.7	Lungs	2.6
Kidney	6.6	Blood	1.3
Heart	4.7	Brain	0.5

Figure 18 illustrates that the ^{32}P content of the soft tissues of the mouse decreases with time. These investigations were carried out by Marinelli and Kenney[37] to determine whether the uptake of ^{32}P by the muscle, bone, and skin of the leg of the mouse is influenced by irradiation with x-rays. In these experiments one leg was irradiated with 3000 r. while the other leg was protected. A negative result was obtained except in the case of the bones (see Chapter X). No difference in the ^{32}P uptake per gram of tissue was detected in irradiated muscle as compared to nonirradiated muscle. The average ratio of the first to the second, as ascertained in 21 determinations involving 84 animals, is 0.95 and the standard error of the mean is 0.05. Neither was a sig-

[36] H. B. Jones, I. L. Chaikoff, and J. H. Lawrence, *Am. J. Cancer*, **40**, 243 (1940).
[37] L. D. Marinelli and J. M. Kenney, *Radiology*, **37**, 691 (1941).
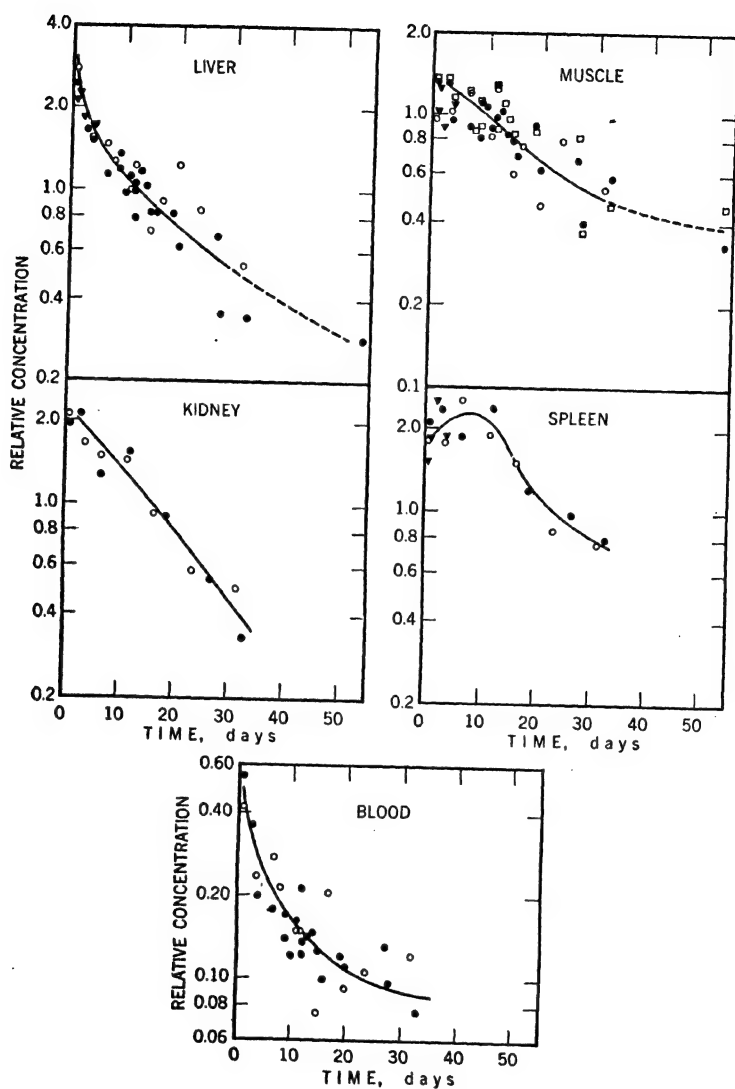
Fig. 18. Relative ^{32}P concentration in tissues[37] of partially irradiated (●) and control (○) mice at different times after administration of the isotope: (▼) represents Berkeley mice; (□) represents values for the nonirradiated muscle of the mice whose irradiated muscle values are shown on the same graph. Ordinates (relative concentration) are the ratio of concentration in tissue to the average concentration of the total animal.

nificant difference found in irradiated skin as compared with non-irradiated skin. (The effect of irradiation on ^{32}P uptake is discussed on page 432.)
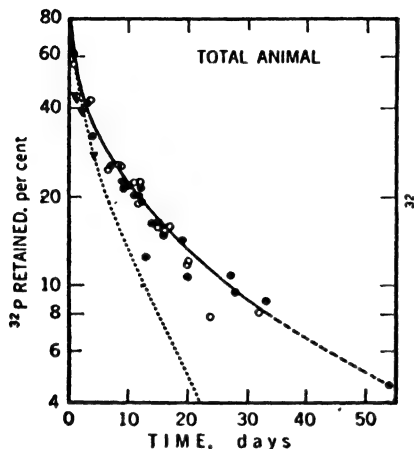
Fig. 19. ^{32}P retention by control (○), irradiated (●), and Berkeley (▼) mice sacrificed at various times after isotope administration.[37] The solid line is the calculated ^{32}P that would have remained in the animal had no radioactive decay occurred; the dotted line represents actual activity.
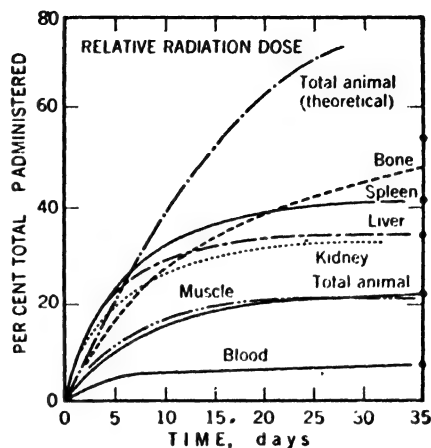
Fig. 20. Radiation dosage due to the beta ray activity of ^{32}P in tissues.[37] The ordinate represents per cent of administered total dose. The bone is seen to obtain an increasing share of the radiation dosage with time.

From Figure 19 we see that mice given labeled phosphate intraperitoneally retain *ca.* 9% of the radioactive isotope after 30 days. In these experiments the relative radiation dosages delivered by ^{32}P to several tissues were computed by determining the concentration of the isotope in these tissues as a function of time (see Figure 20). It was found, as was to be expected, that the rate of elimination of the isotope by a tissue is an important factor in the radiation dose actually received by such tissue.

Scott[38] found that the retention of radiophosphorus by 1 g. mouse muscle tissue, after correction for excretion, was about the same after the lapse of 1 and of 19 days. The retention by the liver declined, while that by the bones increased, as is seen in Table 29.

The behavior shown by muscle tissue can possibly be explained by the low permeability of muscle cells. In spite of a declining ^{32}P content

[38] K. G. Scott, *Cancer Research,* **5,** 365 (1945).

of the plasma, the muscle conserves much of its radiophosphorus acquired during a phase of high plasma ^{32}P content. The easily permeable liver cells lose an appreciable part of their ^{32}P content with time. The marked increase with time of ^{32}P uptake by the bones is due to an almost irreversible incorporation of ^{32}P with the bone apatite crystals.

TABLE 29

^{32}P Retention by Organs of the Mouse[38]

Organ	Per cent of administered ^{32}P retained[a] after						
	1	2	3	4	5	8	19 days
Muscle	1.8	2.0	2.2	2.0	2.4	2.3	2.1
Liver	3.0	3.1	3.2	2.7	3.4	4.2	2.6
Bone	5.5	9.81	5.2	9.0	15.6	15.6	27.2

[a] Values are corrected for excretion.

3. Distribution in the Human

Lawrence and his associates[39] determined the ^{32}P content of the organs of several deceased patients. Some of the results obtained are seen in Table 30. During 74 days prior to death, numerous doses amounting to an aggregate activity of 44 millicuries were administered. The figures indicate that liver phosphorus shows the highest, teeth phosphorus the lowest, specific activity.

Similar results obtained by Erf[40] are seen in Table 31. To the subject investigated a single dose of radiophosphorus was administered 19 days before death due to chronic lymphoid leukemia. Further data on the distribution of ^{32}P in the human organism are to be found in Chapter X.

E. SKIN ABSORPTION OF TRI-*o*-CRESYL PHOSPHATE

When recent technical developments indicated that tri-*o*-cresyl phosphate, $(C_6H_3CH_3OH)_3 PO_4$, may have wider industrial applications, the problem of the routine handling of the material arose, and with it the question of toxic effects from chronic absorption through the skin. Since skin absorption is presumably not of a large magnitude, the use of a radioactive tracer appeared to be singularly useful in attempting

[39] J. H. Lawrence, K. G. Scott, and L. W. Tuttle, *New Intern. Clinics* III, **33**, (1939).
[40] L. A. Erf, *Proc. Soc. Exptl. Biol. Med.*, **47**, 287 (1941).

to answer the questions — how much tri-*o*-cresyl phosphate can be absorbed through the unbroken skin and how rapidly does this absorption occur? This problem has been attacked using tri-*o*-cresyl phos-

TABLE 30

Total and Radioactive Phosphorus in Tissues
of a Lymphatic Leukemia Patient[39]

Tissue	Activity, μc./g.[a]		Total P, mg./g.		Activity, μc./mg. P
	Wet wt.	Ash wt.	Wet wt.	Ash wt.	
Liver.................	0.368		2.09		0.176
Sternal marrow.........	0.338		17.86		0.0189
Aortic lymph node.......	0.318		2.67		0.1191
Sternal marrow.........	0.306		23.6		0.0129
Axillary lymph nodes	0.302		2.41		0.1252
Sternum...............	0.275	1.31	35.0	168.4	0.0078
Spleen.................	0.274		2.02		0.1355
Adrenal gland..........	0.236		1.92		0.1230
Kidney................	0.227		1.78		0.1272
Vertebra...............	0.219	0.892	40.06	165.0	0.0055
Rib...................	0.210	5.65	63.10	167.0	0.0033
Sternal cortex..........	0.176	0.442	64.1	160.9	0.0027
Lung..................	0.175		1.47		0.1190
Heart muscle...........	0.154		1.41		0.109
Skeletal muscle.........	0.142		1.58		0.0901
Thyroid...............	0.094		0.9055		0.1037
Small intestine.........	0.092		0.831		0.1150
Cerebellum.............	0.053		2.78		0.0190
Teeth.................	0.052	0.072	129.5	180.0	0.0004
Brain stem.............	0.039		3.06		0.0127
Blood, whole...........	0.038		0.278		0.1370
Frontal lobe...........	0.037		2.82		0.0131
Sternum[b]...............		0.697		118.7	0.005
Sternal cortex..........		0.590		165.0	0.0036
Vertebra[b]..............		0.586		131.0	0.0043
Rib[b]..................		0.173		106.0	0.0016

[a] μc. = microcurie.
[b] Extracted with sodium hydroxide to remove organic matter.

phate containing radioactive phosphorus, and the absorption has been measured in two human subjects and in a dog.[41]

The two subjects applied weighed amounts of tri-*o*-cresyl phosphate

[41] H. C. Hodge and J. H. Sterner, *J. Pharmacol.*, **79**, 225 (1943).

TABLE 31

Total and Radioactive Phosphorus in Tissues of a Lymphoid
Leukemia Patient[40]

Organ	Total wet wt., g.	^{31}P, mg./g. wet wt.	Activity of whole organs		Activity of organ aliquots, μc./g. wet wt.
			μc./g. wet wt.	μc./.mg ^{31}P	
Aorta.............	122.0	1.25	0.03	0.18	0.02
Bladder					
Gall bladder	14.2	0.39	0.02	0.05	0.03
Urinary bladder ..	86.5	0.38	0.02	0.04	0.02
Bone					
Calverium........	55.5	87.1	0.02	0.0002	0.01
Femur diaphysis..	192.5	92.8	0.04	0.0004	0.01
Femur epiphysis..	441.9	44.4	0.04	0.0007	0.02
Ribs.............	142.0	46.1	0.09	0.0016	0.16
Tibia diaphysis...	123.9	89.5	0.03	0.0003	0.02
Sternum.........	11.0	19.9	0.08	0.0033	0.12
Vertebral bodies (thoracic and lumbar).......	84.3	20.8	0.09	0.0038	0.08
Brain					
Cerebellum......	175.0	2.72	0.03	0.0093	0.03
Cerebrum........	1192.0	1.97	0.03	0.013	0.02
Colon............	393.0	1.46	0.03	0.016	0.03
Cord, spinal	18.6	2.18	0.03	0.01	0.03
Duodenum........	120.5	1.46	0.05	0.028	0.04
Fat, mesenteric.....	61.0	0.30	0.02	0.041	0.01
Eyes.............	17.0	0.51	0.02	0.031	
Ileum	211.0	0.81	0.03	0.032	0.03
Jejunum..........	311.0	1.24	0.03	0.022	0.04
Kidney...........	278.5	1.22	0.06	0.045	0.05
Liver.............	1830.0	1.57	0.09	0.047	0.07
Lung.............	1305.5	1.20	0.04	0.03	0.04
Lymph nodes					
Bronchial........	15.8	1.64	0.08	0.039	0.06
Peripheral.......	34.5	3.08	0.07	0.019	0.06
Marrow					
Tibia diaphysis...	20.5	11.48	0.03	0.0025	0.07
Femur epiphysis..	63.6	26.6	0.04	0.0012	0.03
Femur diaphysis..	37.9	11.29	0.03	0.0026	0.03
Muscle					
Diaphragm......	159.0	1.25	0.03	0.021	0.03
Heart...........	403.0	1.16	0.04	0.029	0.05
Rectus femoris ...	300.5	1.77	0.05	0.022	0.04
Tongue..........	74.0	1.35	0.06	0.036	0.05

TABLE 31 (*continued*)

Organ	Total wet wt., g.	^{31}P, mg./g. wet wt.	Activity of whole organs µc./g. wet wt.	Activity of whole organs µc./mg. ^{31}P	Activity of organ aliquots, µc./g. wet wt.
Pancreas	113.5	1.22	0.02	0.015	0.03
Prostate	16.3	3.21	0.04	0.011	0.04
Skin	228.0	0.82	0.01	0.016	0.01
Spleen	910.0	1.60	0.07	0.035	0.04
Stomach	193.0	1.08	0.04	0.032	0.04
Testes	16.8	1.67	0.06	0.031	0.05
Thyroid	10.3	1.83	0.04	0.017	0.04

containing marked phosphorus to the palms of both hands and rubbed the material briskly until it was evenly distributed. Subject S applied 0.22 g., and subject H 0.11 g. at 8:30 a.m. This sample of tri-*o*-cresyl phosphate had a radioactivity of 1,600,000 counts per minute per 0.1 gram. The tri-*o*-cresyl phosphate was removed at noon of the same day (3.5-hour exposure) by washing the hands in appropriate solvents. The rapidity with which tri-*o*-cresyl phosphate entered the blood stream was striking; subject S had 13 µg. of tri-*o*-cresyl phosphate per 100 milliliters of blood at the end of one hour, subject H had 4, as shown in

TABLE 32

Blood Levels of Tri-*o*-cresyl Phosphate in Humans
Following its Application to the Palms of the Hands[41]

Date (March, 1941)	Hour	Activity of blood sample, counts/min./10 cc. Subject S	Activity of blood sample, counts/min./10 cc. Subject H	Per cent dose/100 cc. blood × 10² Subject S	Per cent dose/100 cc. blood × 10² Subject H	Tri-*o*-cresyl phosphate, µg./100 cc. blood Subject S	Tri-*o*-cresyl phosphate, µg./100 cc. blood Subject H
3/5	8:30 a.m.	tri-*o*-cresyl phosphate applied to palms					
	9:30 a.m.	1.1	0.7	0.52	0.40	13	4
	11:45 a.m.	0.4	0	0.22		4	
	3:45 p.m.	0.2	0.3	0.01	0.18	2	2
	8:30 p.m.	0	0				
3/6	9:15 a.m.	1.4	0.5	1.1	0.68	21	7
3/7	9:00 a.m.		0				

Table 32. These high levels were not maintained, partly due to urinary excretion as indicated by the activity of the urine. Thus, although detectable amounts were still present in the blood 24 hours later, no

radioactivity was found after 48 hours. The urinary excretion of tri-*o*-cresyl phosphate began promptly; subject S excreted 7 μg. in the first hour after application, subject H excreted 10. In subject S, the urinary excretion rate rose to a level of 35-40 μg. per hour; this rate continued for nearly 24 hours. In subject H, no such high values were found; instead the rate varied between 2 and 9 μg. per hour during the first 24-hour period. Forty-eight hours following the application, tri-*o*-cresyl phosphate was still being excreted at rates of 8 and 2 μg. per hour in subjects S and H, respectively. The excretion rate had fallen to 2 μg. per hour in subject S 72 hours after the application. Considering the small absorbing area, the total amounts of tri-*o*-cresyl phosphate excreted via the urine were surprisingly large. Subject S excreted 797 μg. tri-*o*-cresyl phosphate, or 0.36% of the amount applied; subject H excreted 143 μg., or 0.13% of the amount applied.

The investigation of the distribution of ^{32}P following the administration of labeled tri-*o*-cresyl phosphate shows that, after a lapse of 24 hours, the retention per gram tissue takes place in the following order: liver, blood, kidney, lung, heart, spinal cord, and brain.

F. UPTAKE OF RADIOPHOSPHORUS BY GROWING TISSUE

Tissue grown in a labeled medium is bound to contain labeled compounds. Growing tissue, therefore, contains more ^{32}P than fully grown tissue. Resting ovary of rabbits, for example, weighing 0.160 g., takes up only 5 units of labeled phosphorus administered, while the corpus luteum, weighing 0.254 g., in the same experiment shows an activity of 100 units (Bulliard et al.[42]). Tumor tissues show a high and rapid uptake of ^{32}P similar to rapidly growing normal tissues.[43]

The findings of Lawrence and associates[44,45] that the retention of

[42] H. Bulliard, I. Grundland, and A. Moussa, *Compt. rend.*, **207**, 745 (1938); **208**, 843 (1939).

[43] H. B. Jones, I. L. Chaikoff, and J. H. Lawrence, *J. Biol. Chem.*, **133**, 319 (1940). J. H. Lawrence, L. A. Erf, and L. W. Tuttle, *J. Applied Phys.*, **12**, 333 (1941). J. M. Kenney, *Cancer Research*, **2**, 130 (1942).

[44] J. H. Lawrence, K. G. Scott, and L. W. Tuttle, *New Intern. Clinics*, III, 33 (1939). J. H. Lawrence and K. G. Scott, *Proc. Soc. Exptl. Biol. Med.*, **40**, 694 (1939). K. G. Scott and S. F. Cook, *Proc. Natl., Acad. Sci. U. S.*, **23**, 265 (1937). L. Tuttle, K. G. Scott, and J. H. Lawrence, *Proc. Soc. Exptl. Biol. Med.*, **41**, 20 (1939). L. W. Tuttle, L. A. Erf, and J. H. Lawrence, *J. Clin. Invest.*, **20**, 57, 577 (1941). L. A. Erf and G. Friedlander, *Proc. Soc. Exptl. Biol. Med.*, **47**, 134 (1941).

[45] J. H. Lawrence, L. W. Tuttle, K. G. Scott, and C. L. Conner, *J. Clin. Invest.*, **19**, 267 (1940).

^{32}P per gram in various tissues of the leukemic mouse is greater than the retention by the normal mouse (see Figure 21) present another example of the enhanced incorporation of ^{32}P in the growing and rapidly metabolizing tissues. A pronounced selective uptake of ^{32}P by lymph nodes invaded by lymphosarcoma was also found to take place.[46]
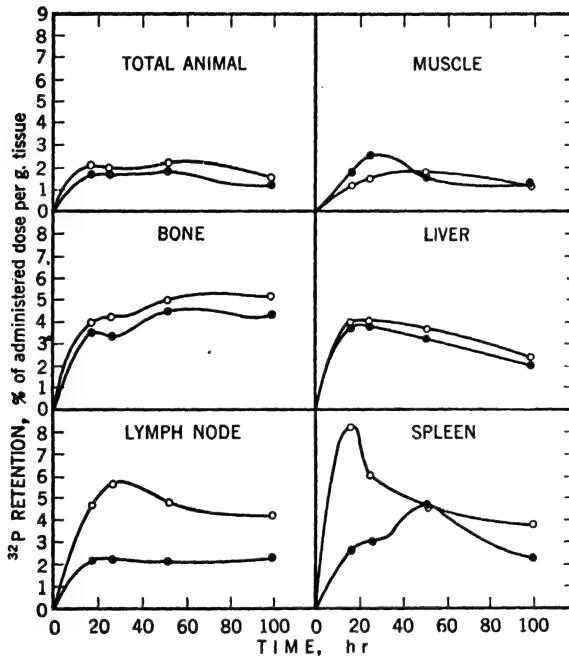
Fig. 21. Distribution of radiophosphorus in the tissues of normal (●) and leukemia (○) mice.[45]

Recent experiments by Scott[47] indicate that lymphomatous tissue retains more radioactive phosphorus than normal tissue 1 to 19 days after administration of labeled phosphorus. In these experiments the retention of ^{32}P in muscle, liver, bone, and lymph nodes of lymphomatous mice was compared with that of similar tissues in normal mice.

Warren,[48] investigating radioactive phosphorus in the tissues of ten patients dead of leukemia, found that tissues which usually show a heavy infiltration of leukemic cells, as the liver, spleen, kidneys, and

[46] J. M. Kenney, L. D. Marinelli, and L. F. Craver, *Am. J. Roentgenol. Radium Therapy*, **47**, 217 (1942).

[47] K. G. Scott, *Cancer Research*, **5**, 365 (1945).

[48] S. Warren, *Cancer Research*, **3**, 334 (1943).

bone marrow, contained relatively large amounts. Radioactive phosphorus, given by mouth to patients with bone tumors, was localized in the sarcoma tissue, which contained the most phosphatase.[49]

When applying ^{32}P as a therapeutic agent in cancer it is important to concentrate a large percentage of the ^{32}P administered in the tumor. In order to determine to what extent ^{32}P accumulates in carcinoma of the breast, osteogenic sarcoma, and lymphosarcoma, Kenney and his associates,[50] after administering labeled phosphate, removed portions of different tissues and determined the ratio in each instance between the amount of the radioactive isotope measured per kilogram of tissue and the amount of ^{32}P administered per kilogram body weight. This ratio has been designated the "differential absorption ratio" and has been used to compare the amount absorbed by the different tissues both in the same patient and in different patients. The "differential absorption ratio" of ^{32}P for patients with carcinoma of the breast is seen in Table 33. Metastatic node is seen to concentrate ^{32}P to an appreciable

TABLE 33

Differential Absorption Ratio of Radioactive Phosphorus
for Patients with Carcinoma of the Breast[50]

^{32}P administered, μc./kg. body wt.	Experimental period,[a] days	Differential absorption ratio							
		Primary tumor	Breast tissue	Metastatic node	Normal node	Muscle	Fat	Skin	Blood
5.6	1	1.3	0.2		1.8	0.7	0.2	0.3	0.8
4.9	2	0.8	0.4		2.4	0.8	0.1	0.3	0.7
2.6	5	1.9	0.3	3.3	2.5	1.2	0.2	0.5	0.5
5.4	5	0.8	0.2		1.3	0.9	0.1	0.2	0.3
9.1	5			4.0	2.8	1.3	0.1	0.4	0.4
5.5	5	1.6	0.3	3.6	1.6	1.0	0.1	0.5	0.1

[a] Days between ^{32}P administration and operation.

extent and both the primary tumor and the normal node to a higher extent than all other organs investigated, with the exception of the most active organs such as liver, kidneys, and intestinal mucosa.

Among the animal tumors generally used in cancer research, the Brown-Pearce rabbit carcinoma possesses a rather outstanding capacity

[49] H. Q. Woodard and N. L. Higinbotham, *J. Am. Med. Assoc.*, **116**, 1621 (1941).
[50] J. M. Kenney, L. D. Marinelli, and H. Q. Woodard, *Radiology*, **37**, 683 (1941).

for forming metastases in almost any part of the body and, thus, affords good opportunities for the study of the distribution of injected ^{32}P in the case of metastatic and normal tissues. Forssberg and Jacobsson[51] found, in their studies on the distribution of intravenously injected labeled phosphate in animals with transplanted Brown-Pearce carcinoma, that the metastases take up on an average as much ^{32}P as the most active organs, such as liver, kidney, spleen, and suprarenal gland, rather independently of their site in the organism. When, however, the uptake of ^{32}P per gram of wet weight of metastases was compared with the uptake of ^{32}P per gram of less active tissues such as diaphragm, pleura, peritoneum, omentum, mesentery, intestines, testis, and lymph nodes, the metastases were found to take up as much as five times more ^{32}P than the corresponding normal tissue. Since bone marrow takes up a similar percentage of ^{32}P per gram of tissue as metastases, the possibility of destroying or stopping the development of already formed metastases of the Brown-Pearce tumor by administering labeled sodium phosphate without damaging vital parts of the body is very restricted. The administration of radiocolloids (see page 124), however, presents such possibilities.

Forssberg[52] also investigated the uptake of ^{32}P by bone metastases. He found that these metastases take up much larger amounts of labeled phosphorus per gram of wet weight than a corresponding weight of fresh bones, as is seen in Table 34. In some cases of bone metastases investigated, the analyses were rather favorable and the possibility of therapy in such cases, without injuring the radiosensitive blood-forming elements during the treatment, is not considered excluded. (Compare also Forssberg et al.[52a])

Since the selective deposition of permissible amounts of radiophosphorus in cancers is not adequate to produce destruction of the tumor when dibasic phosphate is administered orally or intravenously, studies were carried out by Allen and his associates[53] to determine ^{32}P distribution and concentration when injected directly into the tumor (adenocarcinoma) of C57 strain of mice. Various tissues were removed 24 hours later and analyzed. No significant difference in uptake by the

[51] A. Forssberg and F. Jacobsson, *Acta Radiol.*, **26**, 523 (1945).

[52] A. Forssberg, *Acta Radiol.*, **27**, 88 (1946).

[52a] A. Forssberg et al., *Acta Radiol.*, **28**, 391 (1947).

[53] H. Allen, L. H. Hempelmann, and N. A. Womack, *Cancer Research*, **5**, 239 (1945).

experimental animals and those in the control group was found to take place. Skeleton contained 17% per gram, and liver 5% per gram of the ^{32}P administered; the corresponding figure for the tumor was 4.

TABLE 34

Uptake of ^{32}P by Some Organs[52] of a 47-Year-Old Female[a]

Organ	Activity[b]	Organ	Activity[b]
Whole body	51.3	Mammae	
Kidney		Normal	11.5
Normal	276	Tumor	44
Tumor	264	Muscle	76.3
Spleen	278	Ribs	
Liver		Normal	17.8
Normal	381	Tumor	288
Tumor	318	Skull	
Lung		Normal	107
Normal	138	Tumor	330
Tumor	63	Vertebrae	
		Bone infiltered with cancer . .	231
		Tumor necrotic	218

[a] Metastases in vertebral column, ribs, skull, liver, and lungs. ^{32}P was given twice intravenously 2.8 mc., 4/21; 3 mc., 5/5. Died October 5, 1944.

[b] Expressed as 0.001 μc. per gram wet weight.

Scott[54] compared the uptake of ^{32}P by lymphomatous tissue in mice before and after irradiation with Röntgen rays or neutrons. Irradiation with 300 r., or 50 n was found to depress the uptake of ^{32}P by the tumor about 50%, as seen in Table 35. The uptake of ^{32}P by the liver is only

TABLE 35

Effect of Röntgen Rays on ^{32}P Uptake by Some Organs
of Lymphomatous Mice[54]

Organ	^{32}P, per cent of administered per gram, 24 hr. after injection, 32 hr. after irradiation[a]	
	Average of 5 mice	Per cent of control
Tumor	1.85	47.4
Lymph nodes	2.98	61.2
Spleen	3.60	51.6
Liver	3.35	84.8

[a] 300 r. plus lymphoma subcutaneously 21 days prior to irradiation.

[54] K. G. Scott, *Radiology*, **46**, 173 (1946).

depressed to a minor extent by irradiation. Similar results were obtained in experiments in which the mice were irradiated with 50 n.

In the investigation of the retention of atoms of maternal origin in the white mouse, labeled phosphate was administered to a pregnant mouse. Each offspring was found to contain almost the same amount of ^{32}P. By killing the various offspring at different times and comparing their ^{32}P contents, the amount of maternal phosphorus atoms present in the offspring was determined. By breeding three generations of mice, the first generation of which was given ^{32}P, the passage of phosphorus atoms from one generation to the next was followed. Of the ^{32}P content of an offspring of the second generation at birth, 0.6% was found to be present in an offspring of the third generation. We might expect to obtain the same ratio between the ^{32}P content of the third and fourth generation, and so on. Making this assumption, it can be shown that a mouse of the eleventh generation no longer contains a single phosphorus atom present in the first generation.[55]

Marshak[55a] compared the uptake of ^{32}P by the nuclei and the cytoplasm, respectively, of malignant and normal cells. He observed that the nuclei of the malignant cells not only accumulated more labeled phosphorus than the normal cell nuclei, but also that the relative proportion of the administered phosphorus in the nuclei as compared with that in the cytoplasm was much greater in the malignant cells than in normal cells. This metabolic pattern of the nuclei of malignant cells occurred not only in neoplastic tissue, but was characteristic also for rapidly multiplying normal cells (see Chapter VIII).

These results illustrate strikingly the difference in ^{32}P uptake by interchange and by incorporation during growth.

G. DEPOSITION OF ^{32}P IN SUPERFICIAL TISSUES

The deposition of radioactive phosphorus in the superficial tissues of patients suffering from melanoma and undergoing treatment with ^{32}P has been investigated *in situ*. Both the rate of uptake and the rate of elimination in the affected areas were found to differ from those of the normal skin. In the course of time, the activity of melanotic nodules increased with respect to that of the normal skin, according to the work of Marinelli *et al.*[56]

[55] G. Hevesy, in *The Svedberg Jubilee Volume*. Almqvist & Wiksells, Uppsala, 1944, p. 456.

[55a] A. Marshak, *Science*, **92**, 460 (1940).

[56] L D. Marinelli and B. Goldschmidt, *Radiology*, **39**, 454 (1942).

When the radioactivity on the surface of the skin over various types of breast tumors was measured a day or two before surgical removal of tumors later proved to be malignant, that activity was 25% or more above that of comparative normal areas. The measurement over breast tumors later proved to be benign showed less than 25% difference between involved and uninvolved tissue. These results induced Low-Beer[57] to propose the use of a tracer dose of radioactive phosphorus in the diagnosis of breast tumors.

The following examples illustrate the application of the method.[57a] Radiophosphorus (0.5 millicurie) was administered by intravenous injection to a patient with a palpable left breast tumor. After a lapse of 24 hours, the G-M counter placed over the tumor indicated 3002 counts per minute. Over the corresponding area of the right breast only 704 counts were registered; the measurement of the surface activity of the soft tissue of the right forearm indicated 496 counts. The tracer diagnosis indicated the presence of a malignant tumor, a result borne out by histological investigation. In another patient under the same experimental conditions, 824 counts were registered over the right breast tumor, while the corresponding counts over the left healthy breast and over the left forearm were 784 and 1160, respectively. In this case the tracer diagnosis indicated a benign lesion (chronic systiomastitis), a result which was supported by a histological investigation.

H. UPTAKE OF COLLOIDAL CHROMIUM PHOSPHATE BY RETICULOENDOTHELIAL SYSTEM

An appreciable part of most phosphorus compounds introduced into the circulation is taken up by the liver and decomposed. Colloidal chromium phosphate, however, was found by Jones[58] to be taken up to a very large extent by the liver and to remain for at least one year without detected decrement. Anhydrous chromic phosphate was used in this study because of its inertness and apparent lack of toxic effect; it is easily prepared in a fine and even suspension of particles, 1 μ or less in diameter. Radioactive phosphorus ^{32}P was obtained as a solution of disodium phosphate with nonradioactive phosphorus carrier. Every volume of 0.1 N disodium phosphate was mixed with one volume of 0.5 N chromium nitrate, $Cr(NO_3)_3$, and one-third volume of 1.0 N

[57] B. V. A. Low-Beer, *Science*, **104**, 399 (1946).

[57a] B. V. A. Low-Beer, *personal communication*.

[58] H. B. Jones, J. Wrobel, and W. R. Lyons, *J. Clin. Invest.*, **23**, 783 (1944).

sodium acetate. The crystalline hydrate obtained was centrifuged, washed, and recentrifuged. After complete drying at 110° C., the product was heated to 600° C. in the electric furnace. This final treatment resulted in a dark brown, amorphous powder, insoluble in strong acid or alkali, or in aqua regia. The product was transferred to a 50-ml. serum bottle, half filled with pyrex beads. A suitable quantity of isotonic glucose solution was added (1–2 ml. per 100 mg.) and the bottle autoclaved. Rotation of the bottle in a mill for 12 hours, at a speed which allowed the beads to cascade down the sides, resulted in a preparation suitable for intravenous administration. The final product has the appearance of thin clay mud. The very remarkable uptake of the colloid by the liver of mice is seen in Table 36.

Spleen and lungs take up appreciable amounts as well. The percentage recovery of chromic phosphate per gram tissue 5 days after a 1-mg. dose of chromic phosphate decreased in the order: liver, spleen, lungs, femur, lymph nodes, thymus, kidney, intestine, salivary gland, skin, blood, muscle. In the dog and mice, as much as 90% of the total injected chromic phosphate suspension can be recovered from the liver in 4 to 5 days. By making use of strongly active chromium phosphate, intense local radiation can be obtained in the liver. This radiation is of the order of 100 times the concentration in other tissues, except in the lung tissue (see page 126).

Most of the radioactive material remains at the site of injection when insoluble radioactive chromium phosphate is injected in saline suspension around the periphery of transplanted spontaneous mammary adenocarcinoma of mice. These were found to be completely destroyed by this method of interstatial radiation. The mice were found to tolerate less than 1 millicurie at a single injection. About 0.1 millicurie seemed to be the optimum dose for neoplasms less than 2 cm. in diameter. With doses of 0.082 millicurie or more, regression of the tumor was observed.[58a]

In human subjects also, after interperitoneal injection of radioactive chromic phosphate suspension, most of the ^{32}P was located in the liver. The spleen was found to take up almost as much as the liver per gram wet tissue; in some cases (thrombocytopenic purpura, congenital hemolytic icterus) the spleen was found to be more active than the liver.[57a]

[58a] H. Allen, L. H. Hempelmann, and N. A. Womack, *Cancer Research*, **5**, 289 (1945).

TABLE 36

Uptake of Labeled Chromic Phosphate by Mice[58]

Animal No.	Body weight, g.	Duration of experiment	Final body weight, g.	Dose of chromic phosphate			Liver			Spleen			Lung		
				Volume. ml.	Weight. mg.	Activity. μc.	Uptake/ g. tissue, %	Uptake in whole organ, %	Total r. accu- mulated	Uptake/ g. tissue, %	Uptake in whole organ, %	Total r. accu- mulated	Uptake/ g. tissue, %	Uptake in whole organ, %	Total r. accu- mulated
1		24 hours	18.	0.10	0.26	3.75	99								
2	18.0	24 hours	18.0	0.10	0.26	3.75	72								
3	21.5	24 hours	21.5	0.10	0.26	3.75	140								
4	24.0	24 hours	24.0	0.10	0.26	3.75	63								
5	23.0	108 hours	23.0	0.50	1.3	18.8	83								
6	20.5	30 days	20.0	0.50	1.3	18.8	90	81	4,270	49	4.3	1,140	15.7	2.6	88
7	20.5	10 days	16.0	0.50	20.0	230	78	59	39,800	50	4.9	9,650	6.3	0.63	1,470
8	20.0	15 days	19.0	0.25	10	115	57	65	20,800	54	6.2	9,000	9.0	1.4	1,500
9	21.5	15 days	21.0	0.25	10	115	61	77	22,200	58	7.0	9,300	8.0	1.9	1,330
10	20.5	5 days	20.0	0.25	10	265	70	69	24,500	98	11.7	15,800	7.2	1.3	2,070
11	24.0	23 days	18.0	0.25	10	265	70	67	52,300	60	13.1	20,200	12.1	2.7	3,950
12	22.5	23 days	17.0	0.25	10	265	77	72	57,500	50	14.7	17,000	33.5	6.6	11,300

I. PHOSPHORUS UPTAKE BY *TRICHINELLA* AND BY INSECTS

When rats heavily infected 8–10 months previously with *Trichinella spiralis* were fed labeled phosphate, ^{32}P was detected in the encysted larvae as early as 2 hours after feeding. The ^{32}P content increased greatly within the first 24 hours; maximum content was reached on the fourth day.[59]

Phosphorus uptake by the larvae of a small silk-producing moth was studied by Lindsay and Craig,[60] using radiophosphorus as a tracer. Two weeks before the insects were killed, labeled phosphate was administered orally or parenterally. In view of the difficulties involved in the dissection of small insects, the technique of radioautography was applied. From the radioautograph of the section obtained, the ^{32}P is seen to be selectively accumulated (Figure 22), in the walls of the diges-
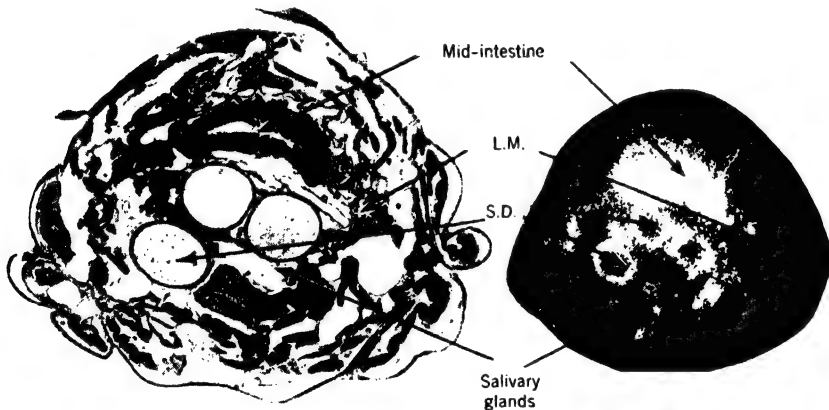
Fig. 22. Transverse section of wax moth larva in region of the mid-intestine. Radioautograph on right shows the distribution of labeled phosphorus.[60] L. M., longitudinal muscle; S. D., silk ducts.

tive tract, the salivary glands, and the ducts of the silk glands. The selective deposition of the labeled phosphorus in these areas indicates that these organs were rapidly developing and, hence, like all growing organs had a greater demand for the administered phosphate.

The rate of mixing of the injected solution with the body fluid was determined for three insects, the yellow mealworm, *Tenebrio molitor* L.,

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[59] O. R. McCoy, V. Downing, and S. N. Van Voorhis, *J. Parasitol.*, **27**, 53 (1941).

[60] E. Lindsay and R. Craig, *Ann. Entomol. Soc. Am.*, **35**, 50 (1942).

the squash bug, *Anasa tristis* (de Geer), and the harlequin cabbage bug, *Murgantia histrionica* (Hahn).

Solutions containing radiophosphorus were injected into the body cavities of the insects, and after varying lengths of time appendages were cut off and tested for radioactive content. By comparing the amount of radioactive substance in the corresponding appendages from each side, cut off at different intervals of time, the time when the injected solution was evenly distributed in the body fluid was found.

This time for *M. histrionica* was 25 to 28 minutes; for *A. tristis*, a little more than 35 minutes; and for *T. molitor*, a little more than 7 minutes.[60a]

J. UPTAKE BY ORGANS OF BACTERIA CONTAINING ^{32}P

Escherichia coli, Staphylococcus aureus, and several other bacteria have been grown on a substrate containing ^{32}P for 18 hours. They were washed and suspended in physiological sodium chloride solution. Then 0.0027 mg. of the bacteria were introduced into the circulation of rats. After the lapse of 30 minutes, the ^{32}P of the different organs was investigated. Highest values were found in the liver and intestine; the lowest in the brain. It is not known which part of the labeled phosphorus present in the organs was due to the uptake of inorganic phosphorus or another phosphorus compound originating from the bacteria introduced into the rats and which part was taken up as a constituent of the labeled bacteria.[61]

Similar investigations were carried out with labeled tubercle bacteria. These were grown for 3 weeks in a Sauton solution containing labeled phosphate; then, a suspension of the bacteria was washed repeatedly with physiological sodium chloride solution and was injected subcutaneously into guinea pigs. After the lapse of 4 hours, fresh lymph gland, per gram of tissue, was found to contain 20 times as much ^{32}P as lung tissue; the corresponding value for spleen was 4, for liver, 1.5.[62]

IV. Arsenic

After injecting labeled arsenic of negligible weight as sodium arsenate into mice, a maximum uptake was found to take place within 1.5 hours;

[60a] R. Craig, *personal communication.*

[61] J. O. Ely, *J. Franklin Inst.,* **232,** 385 (1941); **234,** 500 (1942).

[62] G. Hevesy, K. A. Jensen, and K. Zerahn, *personal communication.*

only in the gonads does the uptake increase further.[63] The distribution of 0.05 mg. of labeled arsenic, injected subcutaneously as As_2O_5, in the different organs of the mouse is seen in Table 37.

Among the organs of the rat the kidneys are found to have the highest arsenic content. A very high arsenic concentration has been found in the red corpuscles.

During the first hour after injecting subtoxic amounts of labeled arsenate into the ear vein of a rabbit, the blood concentration slowly

TABLE 37

Radioarsenic Content of Organs of the Mouse at Different
Times after Subcutaneous Administration[63]

Organ	Radioarsenic per 100 mg. fresh wt., per cent of administered			
	Time after administration, hr.			
	0.75	1.5	3	6
Blood	1.42	1.57	0.84	0.5
Lungs	0.6	0.87	0.66	0.38
Liver	0.062	0.69	0.57	0.23
Kidney	---	3.39	2.43	0.60
Spleen	0.74	0.45	0.53	0.24
Salivary gland	0.26	0.64	0.23	0.17
Gonads	0.03	0.12	0.23	0.17
Brain	0.03	0.08	0.07	0.06

fell; in 168 hours the arsenic had almost disappeared from the circulation. The concentration in the liver, lung, muscle, bone, and tumor rose to a peak rather quickly by the third hour and fell by the twelfth hour.[64]

The distribution of arsenic in the organs varies with the amount administered. Four days after daily administration by subcutaneous injection of about one milligram per kilogram arsenic to rabbits, the distribution seen in Table 38 is found. When investigating the distribution of arsenic in various fractions of mammalian tissue the bulk of arsenic was found in the protein fraction, with a much smaller amount in the acid-soluble fraction and only an insignificant amount in the

[63] H. J. Born and H. Timoféeff-Ressowsky, *Naturwissenschaften*, **29**, 182 (1941).
[64] O. Du Pont, A. Ariel, and S. L. Warren, *J. Applied Phys.*, **12**, 324 (1941).

lipide fraction. In the erythrocytes in which arsenite is concentrated it[66] appears to be bound to the hemoglobin molecule.

TABLE 38

Distribution of Radioarsenic in Organs of the Rabbit[65]

Conditions and tissue	4[a]	Animal number 5	8
Daily As, mg./kg.	0.96	0.99	0.98
Total As, mg./kg.	0.96	0.99	3.91
Killed after last dose, days	1	1	1
Whole blood/cc.	0	0.084	0.069
Liver, μ/g.	0.034	0.39	0.63
Spleen, μ/g.	0.009	0.13	0.22
Kidney, μ/g.	0.01	0.49	0.83
Brain, μ/g.	0.005	0.057	0.10
Bone marrow, μ/g.	0.005	0.15	0.11
Lung, μ/g.	0.05	0.62	0.78
Muscle, μ/g.	0	0 25	0.20
Thyroid, μ/g.	0.02	0.15	0.23
Ovaries, μ/g.	0	0.08	0.14
Uterus, μ/g.	0.01	0.12	0.11
Bile, μ/cc.	0	0.34	0.64
Bladder urine, μ/cc.	0.25	2.75	8.3

[a] Absorption from injection very low.

Human subjects received 1.5 mg. labeled arsenic daily for 4 days. Within 24 hours after administration, 50% of the arsenic was excreted. Of the amount excreted, 99% was found in the urine.[65] Most (99.9%) of the labeled arsenite injected as the potassium salt in humans is excreted through the kidneys. Forty to fifty per cent of a 1.5-mg. dose of arsenic injected on 4 consecutive days is excreted in the course of the first 10 days.[65]

When using carrier-free arsenic very different results are found.[67] After the lapse of 4 days 50% of the [74]As injected intramuscularly as sodium arsenate is present in the red corpuscles of the cat and rat, the amount present in the plasma being negligible. While the skeleton contains 3% or less, the total amount of [74]As present in all other organs

[65] F. T. Hunter, A. F. Kip, and J. W. Irvine, Jr., *J. Pharmacol.*, **76**, 207 (1942),

[66] O. H. Lowry, F. T. Hunter, A. F. Kip, and J. W. Irvine, Jr., *J. Pharmacol.*, **76**, 221 (1942).

[67] H. Lanz, *personal communication.*

is less than 2%. After the first 4 days, the daily excretion amounts to 0.5% of the dose administered.

The uptake of arsenic by the organs of cotton rats naturally infected with *Litomosoides carinii* was investigated.[68] Adult *L. carinii* living in the pleural cavity were found to have a specific affinity for arsenic. The distribution of radioarsenic 24 hours after intraperitoneal injection of 1.6 mg. labeled sodium arsenite to cotton rats is illustrated in Figure 23.
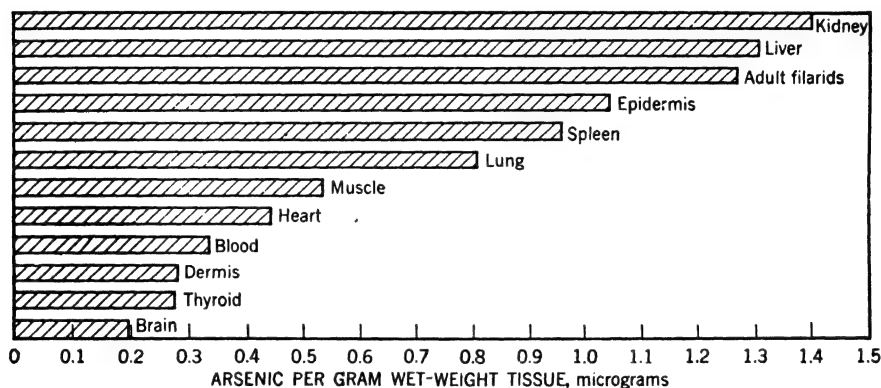
Fig. 23. Average arsenic concentration of tissues of six cotton rats 24 hours after intraperitoneal injection of 1.6 mg. arsenic (as sodium arsenite) per kilogram body weight.[68]

Radioarsenic was not taken up by growing tobacco mosaic virus.[68a]

A comprehensive comparative study of the metabolism of arsenic and phosphate using isotopic tracers is still outstanding. It follows, however, from the work of Lowry and associates[66] that, from the comparative distribution and behavior on hydrolysis of phosphorus and arsenic, there is little evidence for the replacement of phosphorus by arsenic in the tissue. Nor is there evidence that, in contrast to radiophosphorus, arsenic accumulates in rapidly growing tissue.[65]

V. Sulfur and Selenium

After the first 9 hours following administration of 202 mg. labeled sulfur to a human patient, the urine contained 15% of the administered

[68] A. H. Lawton, A. T. Ness, F. J. Brady, and D. B. Cowie, *Science*, **102**, 120 (1945).

[68a] H. J. Born, A. Lang, and G. Schramm, *Arch. Virusforsch.*, **2**, 32 (1943).

dose; in the next 15 hours, 32% was found in the urine. During the next days no radiosulfur was detected in the urine.[69] The turnover of sulfur is discussed in Chapter VIII, where data are also presented on amount of sulfur excreted after the administration of elementary sulfur, sodium sulfide, and organic compounds containing sulfur.

With radioactive selenium as a tagged atom, time–excretion studies of exhaled selenium have been made after subcutaneous injection of sodium selenate in the rat. It was found that, after single subcutaneous injections of selenate (3–4 mg. selenium per kg.), 3 to 10% of the original dose was exhaled as a volatile compound within 24 hours. Approximately 55 to 75% of the selenium was expired by the third and sixth hours, respectively.[70] Data on the distribution of selenium are given by McConnell.[70]

VI. Distribution of Chlorine and Bromine

After gaseous radiochlorine was inhaled by mice, the activity figures shown in Table 39 were found by Born and Timoféeff-Ressowsky.[71] The rapid decrease in the radiochlorine content of the lungs is followed by an increase in the radiochlorine content of the kidneys.

TABLE 39

Relative Activity of Various Organs of the Mouse after Inhalation
of Radiochlorine Gas[71]

Organ	Immediately after inhalation	Eight minutes after inhalation
Lungs	415	93
Kidneys	47	89
Liver	36	13
Brain	27	12

Chlorine and bromine are mainly found in the extracellular fluid. Their distribution will be described in connection with the determination of the extracellular space (see page 201). The distribution of organic compounds containing radioactive bromine is discussed in the following section.

[69] H. Borsook, S. Keighley, D. M. Yost, and E. McMillan, *Science*, **86**, 525 (1937).
[70] K. P. McConnell, *J. Biol. Chem.*, **145**, 55 (1942).
[71] H. J. Born and H. Timoféeff-Ressowsky, *Naturwissenschaften*, **28**, 253 (1940).

VII. Organic Compounds Containing Radiobromine

The total distribution of an injected dose of radioactive dibromo Evans blue 24 hours after injection into the mouse was investigated by Moore et al.[72] Their results are shown in Table 40.

TABLE 40

Distribution of Radioactive Dibromo Evans Blue in Mice[72]

Tissue	Concentration, per cent of dose per gram tissue	Tota. uptake, per cent of dose in entire organ or tissue
Colon...................	18.6	13.0
Liver...................	12.5	16.2
Spleen..................	4.98	1.37
Intestine (small)..........	4.85	7.30
Stomach................	4.81	1.57
Kidneys................	4.60	1.72
Tumor (on diaphragm).....	3.12	2.07
Skin...................	3.12	11.2
Testes.................	2.68	0.70
Brain..................	1.88	0.28
Bladder................	0.69	0.40
Carcass................	———	21.97
Blood..................	2.74	5.45
Bile...................	4.63	0.46
Urine..................	2.61	1.61
Feces..................	30.4	15.2

Labeled 2-bromo-3-hydroxy-1,4-naphthoquinone (containing radioactive bromine) was employed in the study of the blood-clotting effect of this substance. The compound was found to be absorbed at a remarkable rate after subcutaneous injection. The liver takes up only a minor part of the substance.[73] Daudel and co-workers[74] also used radioactive α-bromotriphenylethylene to study the metabolism of synthetic estrogenic substance. In female mice high concentrations could be detected in the ovaries, uterus, and mammae, especially when the experiments were performed on animals in full, spontaneous oestrus.

[72] F. D. Moore, L. H. Tobin, and J. C. Aub, *J. Clin. Invest.*, **22**, 161 (1943).

[73] M. Berger, Ng. Ph. Buu-Hoi, R. Daudel, P. Daudel, and S. May, *Experientia*, **2**, 184 (1946).

[74] P. Daudel, R. Daudel, M. Berger, Ng. Ph. Buu-Hoi, and A. Lacassagne, *Experientia*, **2**, 107 (1946).

The males are characterized by a selective fixation of the estrogen in the preputial glands.

Radioactive dibromotrypan blue offers us a means of diagnosing localized inflammation, for example, the location of abscesses on the legs or in the subcutaneous tissue of the abdominal wall of the rabbit. The radioactive colloidal dye concentrates in inflammatory lesions to an extent detectable from outside the intact animal with a suitable counter. In rats, 0.1 to 0.5 microcurie was a sufficient dose of radioactive dye to give satisfactory readings. For rabbits, 1.5 microcuries was found to be satisfactory. The radioactive dye contained ^{82}Br (half-life $= 34$ hours). The detecting instrument was a Geiger counter screened with lead to focus its registration on one portion of the animal at a time. Using this radioactive dye, lesions in the periphery of the body were found to be detectable in all cases by Moore and Tobin,[75] while abdominal lesions were detectable in only 77% of the cases investigated.

The applications of radioactive bromoproteins are discussed in Chapter VIII.

In the study of the distribution of dibromodiphenyltrichloroethane containing ^{82}Br in adult American roaches, the compound was dissolved in peanut oil and made into an emulsion which was (a) applied to various parts of the bodies of the roaches or (b) injected into the abdomen of similar roaches. The distribution of radioactive material in the bodies was determined after 1–2 hours when toxic symptoms were evident, in case a by use of the Geiger counter and in case b photographically. Penetration is relatively rapid from the femur, the antennae, and the undersurface of the rear wings; penetration is slower from the tarsi, the front wings, and the lower surface of the abdomen. Following the injection, radioactive material during the first hour collects predominately in the region of the stomodeal valve, in the ovaries, and the cerci. Much less is found in the legs, wings, antennae, testes, trachae, thoracic muscles, salivary glands, and ventral nerve cord, and no detectable amount in the Malpighian tubules or fat body.[75a]

VIII. Iodine

A. GENERAL REMARKS

Iodine is found only in minute amounts in the plasma. When administering radioiodine, it is therefore of great importance to employ prep-

[75] F. D. Moore and L. H. Tobin, *J. Clin. Invest.*, **21**, 471 (1942).
[75a] R. Craig, *personal communication.*

arations of infinitesimal weight. Practically weight-free radioiodide can be obtained by bombardment of tellurium with swift deuterons. The metal is dissolved in acid; the iodide is distilled off, and collected in an alkaline solution which is then neutralized and brought to a physiological concentration. The ^{131}I obtained has a half-life of 8.0 days. Tracer doses of ^{131}I weighing only 1.10^{-4} μg. or less have been used. Earlier investigations, however, were carried out with ^{128}I which has a half-life of 25 min. In early animal experiments, the ^{128}I was administered in the form of sodium iodide obtained by dissolving labeled silver iodide in sodium thiosulfate.[76,76a]

More than 70% of a comparatively large amount, 0.5 mg., of iodine administered as potassium iodide to the rat is absorbed at the end of the first hour, as seen from Figure 24. About 13% was eliminated 3 hours after its introduction into the stomach, and 50% at the end of 24 hours. In the figure, the open circles represent absorption, that is, the difference between the amount administered and the amount found in the small intestines. Here, and in Figure 25, each point represents the average of four separate analyses on four animals.

Fig. 24. Absorption and excretion[77] of 0.5 mg. labeled iodine administered as potassium iodide to the rat: O shows absorption, the difference between the amount administered and the amount found in the stomach and small intestines; □, urine; ■, feces.

Uptake and excretion of iodine are largely influenced by the amount administered. This is strikingly elucidated by the fact that, of a dose of radioiodine weighing 0.03 mg., only 7% was taken up by the thyroid gland of the rat, while, of a dose of radioiodine of negligible weight, a tracer dose, the maximum deposition amounted to 65% (see Figure 25). It is the circulation of the tracer dose which claims the chief interest since it reflects the movement of endogenous iodine.[77] In rats and guinea pigs, the daily injection of 0.25 mg. of thyroxine for 11-12 days decreases the ability of the

[76] S. Hertz, A. Roberts, and R. D. Evans, *Proc. Soc. Exptl. Biol. Med.*, **38**, 510 (1938).

[76a] S. Hertz, A. Roberts, J. H. Means, and R. D. Evans, *Am. J. Physiol.*, **128**, 565 (1940).

thyroid to take up radioactive iodine subsequently administered as NaI.[76b]

Plasma proteins iodinated with labeled iodine (^{131}I) were prepared and applied in the study of distribution of plasma proteins.[78]
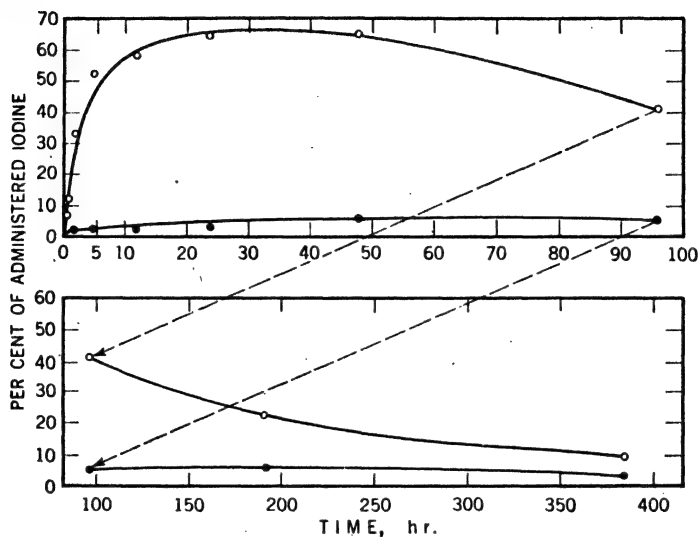
Figure 25. Uptake of labeled iodine by the thyroid gland of the rat,[77] some animals receiving a tracer dose of labeled iodine (○) and others 0.03 mg. labeled iodine (●).

B. DISTRIBUTION

The distribution of tracer doses of radioiodide in organs of the rabbit other than thyroid was studied by Perlman and associates[77] (Table 41). In the early phase of the experiment, kidney shows the highest ^{131}I content. Liver and muscles are found to lose the greater part of their initial ^{131}I content in the course of 197 hours. Evidence is presented suggesting that only ionized iodine can be withdrawn from the blood stream by the thyroid gland. The formation of diiodothyrosine and thyroxine and the role of iodine in thyroid metabolism are discussed in Chapter VIII. Investigation of distribution of radioiodine in the organs of the rabbit following intravenous injection shows that maximum

[76b] F. Joliot, R. Courrier, P. Süe, and A. Horeau, *Compt. rend. soc. biol.*, **139**, 657 (1945).

[77] I. Perlman, I. L. Chaikoff, and M. E. Morton, *J. Biol. Chem.*, **139**, 433 (1941).

[78] J. Fine and A. M. Seligman, *J. Clin. Invest.*, **22**, 285 (1943).

retention is obtained in all organs, except the thyroid, after the lapse of about 5 hours.

TABLE 41

Distribution of Tracer Dose of Radioiodine in Rabbit Tissues
Other Than Thyroid[77]

Tissue	Per cent administered[a] labeled $I \times 1$)^2				
	Time after injection, hr.				
	5	25	48	96	197
Kidney	8.10	0.592	0.539	0.400	0.207
Blood	5.57	0.544	0.421	0.262	0.248
Skin	3.68	0.281	0.222	0.179	0.131
Testes	2.47	0.605	0.576		0.228
Liver	2.43	0.289	0.158	0.117	0.0037
Adrenals	1.84	0.390	0.273	0.070	0.114
Muscle (gastrocnemius)	1.05	0.073	0.056	0.050	0.038
Brain		0.069	0.029	0.016	0.0097

[a] Each animal received subcutaneously an isotonic solution of sodium chloride containing tracer amounts of radioiodine.

When equivalent amounts of labeled thyroxine and sodium iodide, respectively, were injected into the veins of the rabbit, a much greater percentage of labeled thyroxine than of labeled iodide was excreted through the kidneys.[79] The fate of injected labeled thyroxine is discussed in Chapter VIII.

After administering large doses of ^{131}I to rabbits and dogs, Hamilton and Lawrence[80] found almost complete destruction of the thyroid gland without evidence of damage to other tissues of the body.

The distribution of radioiodine dissolved in sodium sulfate injected into the veins of the rabbit was investigated as well,[81] and data are available also on the distribution of radioiodine in the dog.[82] The method of isolation and determination of radioactive iodine in the tissues involves the oxidation of all the iodine in the tissue to iodate by ashing with chromic acid. This is followed by reduction of the excess chromate to the chromic state and of the iodate to iodide by means of

[79] F. Joliot, *Proc. Roy. Soc. London,* **A184,** 1 (1945).
[80] J. G. Hamilton and J. H. Lawrence, *J. Clin. Invest.,* **21,** 624 (1942).
[81] I. Ariel, W. F. Bale, V. Downing, H. C. Hodge, W. Mann, S. N. Van Voorhis, S. L. Warren, and H. J. Wilson, *Am. J. Physiol.,* **132,** 346 (1941).
[82] W. Mann, C. P. Leblond, and S. L. Warren, *J. Biol. Chem.,* **142,** 905 (1942).

sulfur dioxide.   After the excess sulfur dioxide is boiled off, an excess of iodate is added, and the resulting iodine is extracted with carbon tetrachloride.   In order to obtain the sample, the iodine is reduced with sodium thiosulfate and precipitated on hardened filter paper as silver iodide.[77,82]

C. UPTAKE BY RABBIT THYROIDS

Hertz and associates[83] found that the normal rabbit thyroid could collect up to 80 times the quantity to be expected from uniform diffusion throughout the body tissues.   In hyperplastic thyroids, indeed, this relative concentration could reach several hundredfold.   After intravenous injection of radioiodine, the percentage of any dose collected was found to reach a maximum within 10 to 15 minutes, and periods as long as an hour and a half did not increase greatly the amount trapped.   These investigators could detect iodine in the thyroid of animals which died within 2 minutes after injection of the radioactive material.   It was also shown that in normal glands the percentage collected increases with decreasing doses.   Thus, the gland operates more efficiently when less iodine is supplied.   If a thyroid has previously been saturated with iodide, a second injection will not yield much further increase.   A normal rabbit, after receiving a dose of about 50 mg. iodine, is able on the second injection to accumulate only half the labeled iodine which otherwise normal untreated animals would trap.   These early experiments were mostly carried out with ^{123}I having a half-life of 25 minutes only, however ^{126}I, ^{130}I, and ^{131}I were used later.

A detailed survey of those and other experiments published up to 1940 is given by Salter.[84]   The formation of radioactive thyroxine following the uptake of radioiodine by the thyroid is discussed in Chapter VIII.

D. UPTAKE BY HUMAN THYROIDS

Various methods were employed by Hamilton and Soley[85,86] in the study of iodine metabolism of patients suffering from thyroid diseases. In early experiments, labeled iodide was administered to human subjects.   The kidneys eliminated 53–58% in the course of 48 hours, and

[83] S. Hertz, A. Roberts, J. H. Means, and R. D. Evans, *Am. J. Physiol.*, **128,** 565 (1940).

[84] W. T. Salter, *The Endocrine Function of Iodine.* Harvard Univ. Press, Cambridge, Mass., 1940, p. 351.

[85] J. G. Hamilton and M. H. Soley, *Am. J. Physiol.*, **127,** 557 (1939).

[86] J. G. Hamilton and M. H. Soley, *Am. J. Physiol.*, **131,** 135 (1940).

12.5 to 15.4% in the course of the next 72 hours. The feces excreted in 5 days contained 0.08 to 16.7% of the labeled iodide, while in the thyroid glands 0.07 to 17.5% was found after the lapse of 5 days.

In other experiments, the uptake of iodine by the thyroids was meas-
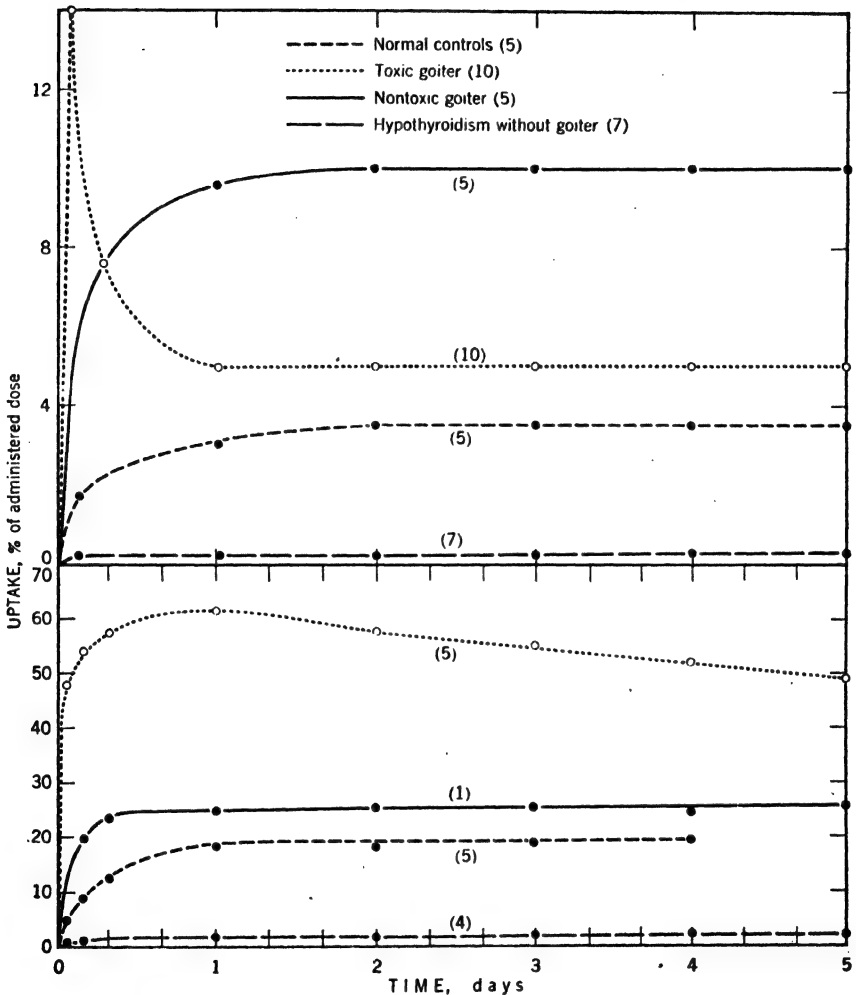
Fig. 26. Radioiodine uptake by intact thyroid glands of normal human subjects and of patients with different types of thyroid disorders.[88] In the experiment shown by the upper graph, each subject received 14 mg. iodine, labeled with radioiodine, as sodium iodide; in the experiment shown by the lower graph, only 0.1 μg. iodine as labeled sodium iodide was given. Numbers in parentheses represent number of subjects.

ured by placing a Geiger counter tube against the gland, a method also applied in experiments by Hertz.[87]   The counter tube employed was 10 cm. long and 1.5 cm. in diameter; for mechanical protection it was encased in a copper tube whose walls were 1 mm. thick.   The counter was used for measurement of the γ-rays emitted by the radioiodine atoms (^{131}I, half-life = 8.0 days) taken up by the thyroid gland.   When a solution of labeled sodium iodide containing 14 mg. of iodine in 100 ml. water was administered to each subject 1 hour before breakfast, the thyroid gland of 5 normal subjects investigated took up 1–5% of the iodide administered, the maximum uptake being reached after the lapse of 1 or 2 days.   The activity of the gland did not decline in the course of the experiment,[88] which lasted 5 days (see Figure 26, upper graph).

Curves of the radioiodide uptake of the thyroids of 2 patients with nontoxic goiters and normal basal metabolic rates were similar in form to those observed for normal subjects.   The only significant difference was that the percentage of radioiodine concentrated in the thyroids of these patients was about 3 times greater than in the normal group.

Curves obtained from patients with thyrotoxicosis differed strikingly from those of the above-mentioned groups. The greatest concentration (7–20% of the amount administered orally) of radioiodine in the thyroids of these patients occurred in 1 to 4 hours after its administration. The concentration rate of the radioactivity fell rapidly and at the end of the first day it was 50–20% of its maximum value.   The content of radioiodine did not change significantly during the next 4 days.   The patients with hyperthyroidism, and also a patient with a goiter and hypothyroidism, took up iodine much more rapidly, but were unable to retain it.

The experiments described above were repeated[88] with a smaller group of patients and normal controls under the same conditions, with the exception that each subject received a test dose which contained a total of approximately 0.1 μg. of iodine, only.   The uptake curves for this group are shown in the lower graph of Figure 26.   A marked increase in the uptake occurred in all four clinical types.   This result was expected, since the total iodine content of the thyroid is relatively small and its capacity to accumulate large amounts of iodine obviously is limited.   In patients with hyperthyroidism the uptake curves failed to repeat the form they took in the previous study.

[87] S. Hertz, *Am. J. Roentgenol. Radium Therapy*, **46**, 467 (1941).

[88] J. G. Hamilton, *Radiology*, **39**, 541 (1942).

The rates of urinary excretion per day of iodine in a group of patients with various types of thyroid disorders differed considerably from the rates for normal controls, as seen in Figure 27.
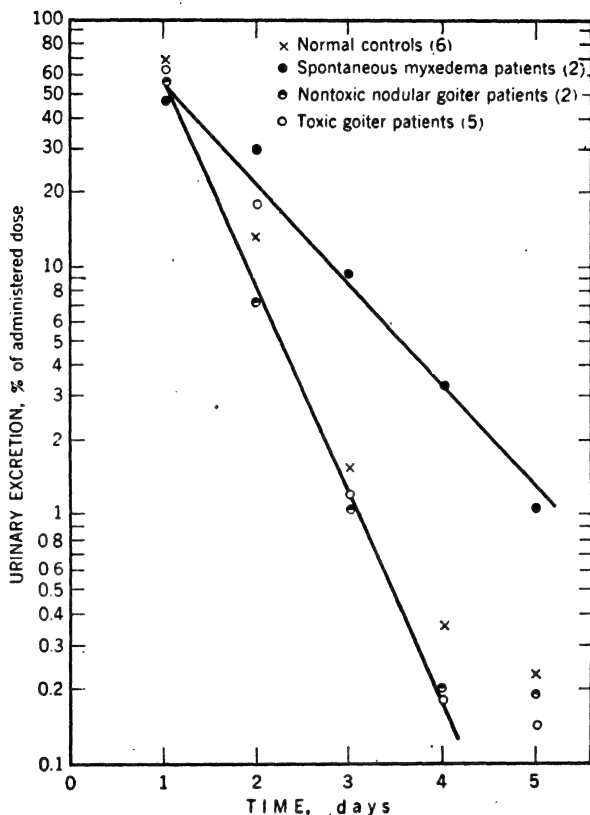
Fig. 27. Urinary iodine excretion rate per day in controls and thyroid patients after administration of 14 mg. iodine as labeled sodium iodide.[86]

Radioactive iodine has also been used in studies of childhood hypothyroidism.[89]

E. GRAVES' DISEASE

Radioactive iodine was used by Hertz and his colleagues[90] as a tracer in the study of iodine metabolism in Graves' disease. The large

[89] J. G. Hamilton, M. H. Soley, W. A. Reilly, and K. B. Eichhorn, *Am. J. Diseases Children*, **66**, 495 (1943).

[90] S. Hertz, A. Roberts, and W. T. Salter, *J. Clin. Invest.*, **21**, 25 (1942).

percentage uptake from a small dose is even more striking in studies on Graves' disease than in experiments on rabbits whose thyroids were made hyperplastic with anterior pituitary thyrotropic hormone.[91] The hyperplastic thyroid of Graves' disease may at first collect 80% or more from a small dose (2 mg). With larger doses, however, this balance is incomplete and iodine finds its way to other organs.

While little if any loss of iodine occurs in normal persons within the first few days following administration of iodine, in untreated cases of Graves' disease, which take up large amounts of iodine, a definite loss of iodine from the gland was found during this period. Thus, the thyroid in Graves' disease does not tend to become saturated with iodine by accumulating small amounts, but tends rather to take up iodine and later to secrete it.

As the ophthalmopathic type of Graves' disease patient excretes more iodine from a 2-mg. (or smaller) test dose than a patient suffering from classic Graves' disease; the urinary iodine excretion can be used as a diagnostic aid in distinguishing the two types of disease.[92]

Radioautographs of Thyroid Sections. Two days after the administration to human subjects of 15 mg. labeled I, having an activity of 0.1 to 1 millicurie, the thyroids were extirpated and sections were prepared from the glands. These sections were placed against photographic films on which the β-radiation from the accumulated radioiodine produced areas of darkening. These images made a pattern of the distribution of the stored radioiodine in the sections of thyroid tissue. The areas of hyperplasia in all types of thyroid tissue examined have the greatest ability to concentrate administered radioiodine. Beautiful photographs were obtained by Hamilton and associates.[93]

Figure 28 (top) was obtained from a sample of normal thyroid tissue; the radioautograph indicates that the accumulated radioiodine was apparently evenly distributed throughout the section. The bottom autograph was obtained from hyperplastic thyroid tissue. It reveals a marked degree of cellular activity, and little colloid is present. The radioautograph indicates that the small amount of remaining colloid has a much higher proportion of the accumulated radioiodine than the cells of the adjacent acini. This observation suggests that, when a

[91] S. Hertz and A. Roberts, *Endocrinology*, **29**, 82 (1941).

[92] S. Hertz and A. Roberts, *J. Clin. Invest.*, **21**, 31 (1942).

[93] J. G. Hamilton, M. H. Soley, and K. B. Eichhorn, *Univ. Calif. Berkeley Pubs. Pharmacol.*, **1**, No. 28 (1940).

marked degree of thyroid hyperplasia takes place, part of the iodine accumulated in the thyroid cells moves rapidly into the colloid, while the remainder is returned by the cells into the blood stream.[88] Figure 29 shows a photomicrograph and radioautograph of a section of tissue from a patient with nontoxic goiter.
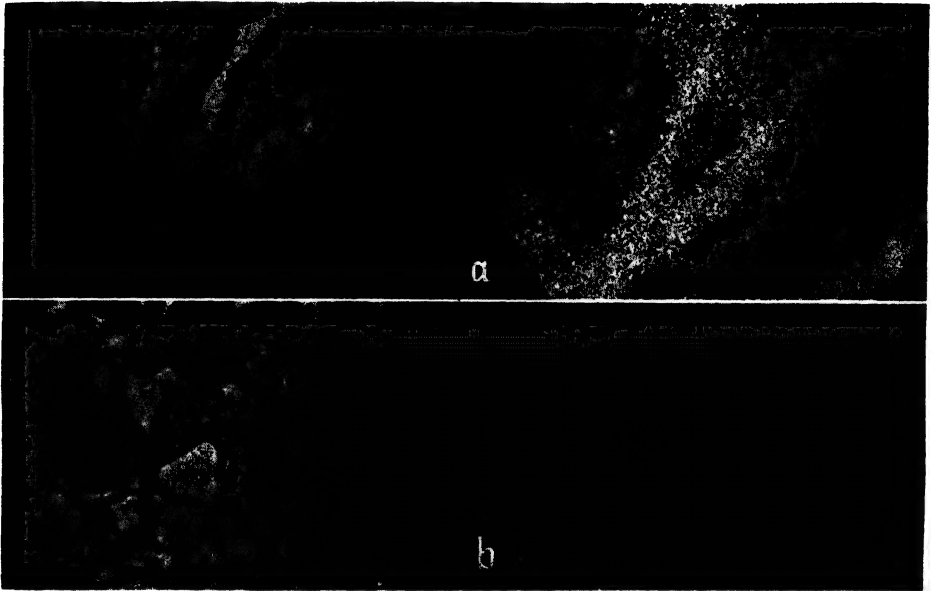
Fig. 28. Photomicrographs (left) and radioautographs (right) of sections of normal (a) and hyperplastic (b) thyroid tissue.[88] The areas of darkening of the radioautographs represent the regions of greater accumulation of radioiodine in the accompanying sections of thyroid tissue. (For part b: the large grayish areas in the section of the left represent colloid, the remaining acini being devoid of colloid. The radioautograph indicates that most of the accumulated radioiodine was stored in the colloid.)

A new photographic technique was recently described by Leblond and co-workers.[93a]

F. UPTAKE BY TUMOR TISSUE

In the study of the localization of radioiodine in cancerous thyroid tissue, no significant deposition of labeled iodine in the malignant areas of the thyroid was observed by Hamilton and Soley[88] in three patients.

[93a] L. F. Bélanger and C. P. Leblond, *Endocrinology*, **39**, 8 (1946). C. P. Leblond, M. B. Fertman, I. D. Puppel, and G. M. Curtis, *Arch. Pathol.*, **41**, 510 (1946).

The thyroids were removed 2 days after administration of tagged iodine. The total iodine content and the proportion of the accumulated labeled iodine were then determined. The results obtained are seen in Table
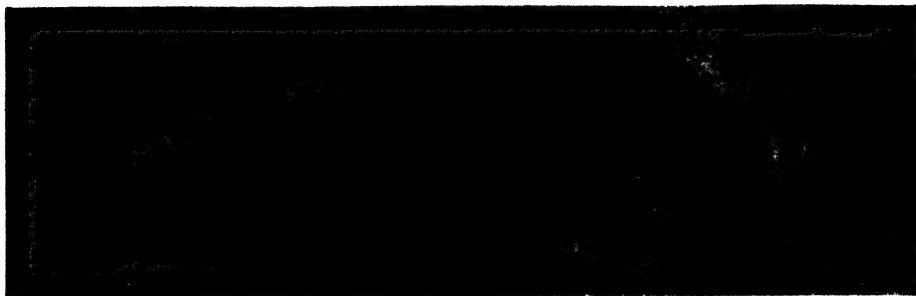
Fig. 29. Photomicrograph (left) and radioautograph (right) of a section of thyroid tissue ($\times$ 60) from a patient with nontoxic goiter.[88] The acini are enlarged and distended with colloid which had accumulated very little of the administered radioiodine. The cells and small acini surrounding the large colloid deposits had a much greater ability to store the labeled iodine.

Fig. 30. Photomicrograph (left) and radioautograph (right) of a section of cancerous thyroid tissue.[88] The diffuse cellular area covering the right half of the section is made up of cancerous thyroid tissue; to the left are three small islands of uninvaded thyroid tissue which accumulated most of the radioiodine.

41A. These data and the radiographs shown in Figures 29 and 30 indicate that the malignant portions of the thyroid have no significant capacity either to take up or to retain iodine. In a recent work, Marinelli et al.[93b] found that certain types of thyroid carcinoma do possess the ability to accumulate radioactive iodine.

[93b] L. D. Marinelli, F. W. Foote, R. F. Hill, and A. F. Hocker, *Am. J. Roentgenol. Radium Therapy*, **58**, 17 (1947).

More radioiodine, from an oral therapeutic dose, was found by Keston *el al.*,[94] to have been taken up by the metastases in the right lower femur (30%) than by the thyroid gland itself (6%). Other metastases which had previously been irradiated with Röntgen rays failed to take up radioiodine in appreciable amounts. The material present in the femoral metastasis and in the thyroid gland could not be washed out of these tissues by administration of 54 mg. potassium iodide. About 3 weeks after the therapeutic dose, the metastasis had lost about 85% of the radioiodine (10 millicuries), while the thyroid still contained about the same amount as originally taken up. A tracer dose given a few days after this showed prompt uptake by the thyroid gland, but no appreciable uptake by the femoral metastasis. The latter was presumably affected by the radiation emitted by the therapeutic dose of radioiodine previously administered.

· TABLE 41A

Radioiodine Uptake by Thyroids and Tumor Tissue of Two Patients with Thyroid Carcinoma after Oral Administration of 14.0 Mg. Iodine Containing Labeled Iodine[88]

Diagnosis	Total tissue weight, g.	Total tissue iodine, mg.	Radioiodine uptake in tissue, %	Iodine per g. tissue, mg.	Radioiodine uptake per g. tissue, %
Carcinoma of Thyroid					
Thyroid tissue	5	1.2	1.2	0.24	0.24
Cancerous tissue	138	<0.2	0.13	<0.002	0.001
Regional metastases . .	139	<0.2	0.05	<0.002	0.0004
Carcinoma of Thyroid					
Normal thyroid tissue .	20	9.0	4.5	0.45	0.23
Cancerous tissue	90	<0.2	0.2	<0.002	0.0022

Seidlin *et al.*[94a] studied some cases of metastatic carcinoma of the thyroid without clinical hyperthyroidism. They found a selective localization of radioiodine in the lesions.

G. APPLICATION IN THE STUDY OF EMBRYOLOGIC DEVELOPMENT OF THE THYROID

Radioiodine with the aid of radioautography has been found a useful tool for the study of the embryologic development of the thyroid

[94] A. S. Keston, R. P. Ball, V. K. Kneeland Frantz, and W. W. Palmer, *Science,* **95,** 362 (1942).

[94a] S. M. Seidlin, L. D. Marinelli, and E. Oshry, *J. Am. Med. Assoc.,* **132,** 38 (1946).

gland. Gorbman and Evans[95] undertook to determine at what stage in the development of the tadpole of the frog, *Hyla regilla,* the thyroid gland possessed the ability to accumulate iodine. The tadpoles, which ranged in size from the freshly hatched larvae 7 mm. in length to 38-mm. larvae with well developed hind legs, were maintained for 2 days in
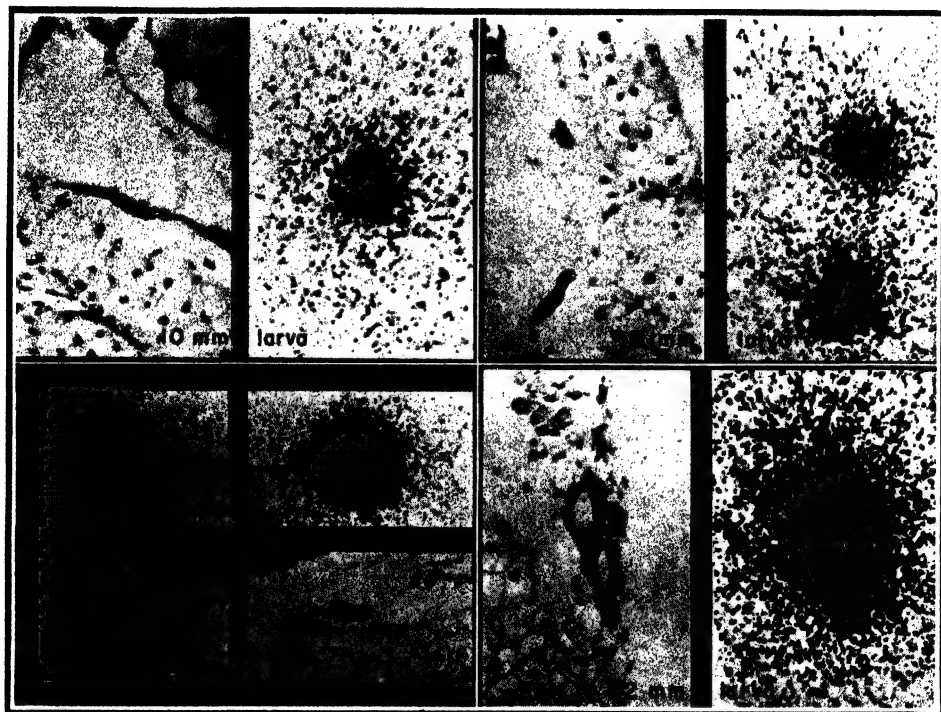
Fig. 31.   Radioautographs of sections of the developing thyroids of tadpoles at various stages of development. The progressive increase in size and development of the thyroids is paralleled by a corresponding increase of their ability to accumulate the labeled iodine, as is indicated by the radioautographs.[95]

800 ml. water which contained 150 microcuries radioiodine and approximately 1 μg. iodine as sodium iodide. At the end of the 2 days the tadpoles were fixed and serially sectioned. Radioautographs were made from the sections, which were later stained with hematoxylin and eosin. A typical series is shown in Figure 31, representing the various stages of development of the tadpole. No evidence of iodine storage

[95] A. Gorbman and H. M. Evans, *Proc. Soc. Expll. Biol. Med.,* **47,** 103 (1941).

could be demonstrated in the larvae whose total length was less than 10 mm. The first evidence of iodine deposition was noted in the 10-mm. larvae. As the tadpoles increased in size, the thyroid became larger, and the follicles, which were made up of more cells and contained greater amounts of colloid, increased in number. These developmental changes were accompanied by a parallel increase in size and intensity of the radioautographs which indicated an increasing ability of the thyroids to accumulate labeled iodine. The labeled iodine in the thyroids of the tadpoles was probably bound in organic combination as thyroglobulin before the radioautographs were taken, for the sections had been treated with various solvents which would have extracted any inorganic iodine in the tissues.

H. STORAGE BY *PEROPHORA ANNOCETENS*

The thyroid gland, engaged in hormone synthesis, is the only organ in vertebrates capable of withdrawing considerable amounts of iodine from the blood stream and storing it within itself in relatively high concentration. The protochordates lack a thyroid gland, however, possessing an organ considered a morphologic homologue, the endostyle. To investigate whether the endostyle of *Perophora annocetens* stores iodine, Gorbman and co-workers[96] placed some of these littoral tunicates in sea water containing less than 0.1 mg. labeled iodide. After the lapse of 2 days, some of the animals were dried on glass slides; others were fixed in formalin and sectioned serially. The slides were placed in close contact with a sensitive x-ray film. After having obtained a satisfactory radioautograph of the serial sections, they stained these sections with hematoxylin–eosin. The radioautographs showed that no tissue within the body proper of the tunicates stored iodine and the tissue responsible for the remarkably strong iodine storage was the stolonic septum. The stolonic septum is a reproductive organ contributing to the formation of the bud. Radioiodide was localized in the endostyle of *Entophenus lamottenii*.[96a]

IX. Distribution of Astatine

The uptake of element 85 (astatine, eka-iodine) was studied at the time of its discovery by Corson, MacKenzie, and Segrè.[97] This radio-

[96] A. Gorbman, *Science*, **94**, 192 (1941).

[96a] A. Goibman and C. W. Creaser, *J. Exptl. Zoöl.*, **89**, 391 (1942).

[97] D. R. Corson, K. R. MacKenzie, and E. Segrè, *Phys. Rev.*, **58**, 672 (1940).

element, no stable isotope of which is known, has a half-life of 7.5 hours; it differs from most other artificially produced radioelements in that it emits α-particles. It is prepared by bombarding bismuth with extremely energetic α-particles (32 m.e.v.) and heating the bismuth after bombardment to 400° C. The volatilized element 85 is collected upon a cold piece of glass suspended above the molten bismuth.

In the study of the circulation of astatine, Hamilton and Soley[98] used guinea pigs 4–5 weeks in age, whose weights ranged from 170–240 g. Daily injections of thyrotropic hormones were given to two-thirds of the animals for a week before the radiohalogens were administered. Equal quantities of radioiodine and astatine were administered by subcutaneous injection to thyrotoxic animals and normal controls. The astatine content in the samples was determined with an ionization chamber which records α-particles only. The content of radioiodine was determined several days later; at that date the astatine had decayed to a negligible quantity.

The results recorded in Table 42 show that astatine is accumulated in the thyroid gland and excreted in a manner similar to iodine. The

TABLE 42

Uptake by Thyroid Gland and Urinary and Fecal Excretions of Radioiodine and Astatine in Normal and Thyrotoxic Guinea Pigs[98]

Animal	Time after administration, hr.	Uptake by thyroid, per cent of administered dose		Urinary excretion, per cent of administered dose		Fecal excretion, per cent of administered dose
		^{131}I, %	Astatine, %	^{131}I, %	Astatine, %	^{131}I, %
Thyrotoxic...	3	26.1	4.2	13.0	16.0	1.0
Normal......	4	8.5	3.4	12.4	8.8	0.8
Thyrotoxic...	18	38.3	10.7	25.7	34.0	5.7
Normal......	18,	16.9	5.4	37.2	36.0	17.0

variations in uptake of radioiodine and astatine by the thyroid glands of the different groups of animals were smilar in these experiments; although the uptake of astatine was considerably less, the proportion of astatine accumulated by the thyroids remained about one-fourth the value of the uptake of radioiodine.

[98] J. G. Hamilton and M. H. Soley, *Proc. Natl. Acad. Sci. U. S.*, **26**, 483 (1940).

Since astatine emits penetrating Röntgen rays, it was possible to study the accumulation in the thyroid gland *in situ* by means of the technique employed to measure accumulation of iodine. A solution containing astatine was administered to a patient with a nontoxic goiter and the uptake was followed by measuring the radioactivity of the thyroid *in situ*. The uptake curve (see Figure 32) resembles closely
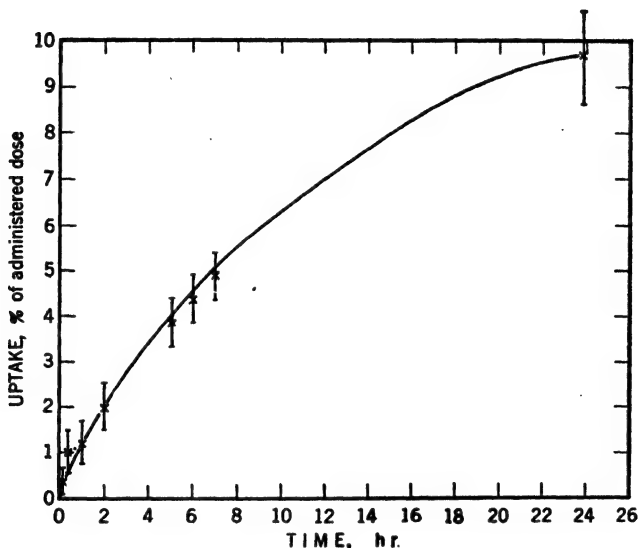
Fig. 32. Uptake of astatine (element 85) in thyroid of a patient with nontoxic goiter.[99]

those obtained with labeled iodine in this type of thyroid disorder; at the end of 24 hours, approximately 10% of the administered sample was accumulated in the thyroid.[99]

Not only the higher, but also the lower homologue of iodine — bromine — was found to be preferentially taken up both by normal and hypertrophic thyroid glands, 1 g. fresh, normal thyroid tissue taking up about 5 times as much radiobromine as 1 g. fresh liver tissue.[100] It may be of interest to investigate the percentage uptake of radiochloride by the thyroid gland.

[99] J. G. Hamilton, *Radiology*, **39**, 554 (1942).

[100] I. Perlman, M. E. Morton, and I. L. Chaikoff, *Am. J. Physiol.*, **134**, 107 (1941).

X. Sodium

Ten minutes after administration of 10 mg. labeled sodium chloride to fasting rats, only 25% of the sodium was present in the contents and tissues of the stomach and the small intestine. About 95% of the labeled sodium had disappeared from the gastrointestinal tract within one hour. With doses of between 0.5 and 20 mg. labeled sodium chloride given by stomach tube in 5 ml. water, the rate of urinary excretion was independent of the size of the dose, about 8% being excreted in the course of the first day. A much larger fraction of the administered sodium was excreted when 100 mg. sodium chloride in 5 ml. water was administered, 18–26% having been excreted in the course of the first day.[101]

In view of the great permeability of the capillary wall to sodium (see page 193) and the fact that most of the body sodium is found in the extracellular fluid (see page 204), administered labeled sodium rapidly comes into exchange equilibrium with most of the sodium present in the organism. Sodium is excreted almost exclusively through the kidneys. The specific activity of urine sodium being equal to that of plasma sodium, assuming constant daily total sodium excretion, the decline of the urine concentration of administered ^{24}Na follows an exponential curve. In the early phase of the experiment, however, in view of the high permeability of the kidney cells, a comparatively large percentage of ^{24}Na will be excreted. A similar behavior is shown by many other substances, for example, by heavy water.[102] Closer investigation of the excretion of labeled substances immediately after intravenous injection may be of interest.

A human subject kept on a normal diet excreted through the kidneys in the course of 67 hours 12.5% of the labeled sodium taken up.[103] The presence of labeled sodium in the urine can be detected 10 minutes after administration of the ^{24}NaCl, as seen in Table 43. (Compare also Süe and Varangot.[103a])

Hamilton[104] studied the rate of absorption of sodium by fasting

[101] D. M. Greenberg, W. W. Campbell, and M. Murayama, *J. Biol. Chem.*, **136**, 35 (1940).

[102] G. Hevesy and E. Hofer, *Klin. Wochschr.*, **13**, 1524 (1934).

[103] G. Hevesy, *Acta Physiol. Scand.*, **3**, 123 (1942).

[103a] P. Süe and J. Varangot, *Compt. rend. soc. biol.*, **139**, 1000 (1945).

[104] J. G. Hamilton, *Proc. Natl. Acad. Sci. U. S.*, **23**, 521 (1937). J. G. Hamilton and R. S. Stone, *Radiology*, **28**, 178 (1937).

human subjects following the oral administration of the radioactive isotope. Absorption of the radiosodium was observed to begin within a few minutes and was apparently complete in from 3 to 10 hours in some subjects, but in others complete absorption was not reached at the end of 10 hours. In infants, most of the radiosodium administered by mouth

TABLE 43

Excretion of Orally Administered ^{24}Na through the Kidneys of a Human[103]

Time after administration	Urine sample vol., ml.	^{24}Na in sample, per cent of administered
10 min..................	5	0.00046
20 min..................	3	0.0076
46 min..................	4	0.036
98 min..................	10	0.056
6 hr....................	80	0.31
19 hr...................	590	2.86
43 hr...................	1700	4.75
67 hr...................	1440	4.5
Total...............	3832	12.5

is absorbed within the first 8 minutes.[105] The fate of ^{24}Na injected into the circulation is discussed on page 193. Radiosodium can be detected in human milk within 20 minutes after ingestion of the isotope. Its peak concentration in the milk is reached in about 2 hours.[106] Labeled sodium was also used to demonstrate the absorption of sodium chloride from fatty bases through the unbroken skin. Radioactive sodium injected intravenously into dogs appears in the pancreatic secretion within 3 minutes, the concentrations of labeled sodium in the juice closely following those of the serum.[107]

^{24}Na moves in both directions across the intestinal epithelium at measurable rates. Even when there is movement of sodium from the gut to the blood against large concentration gradients, small amounts of sodium ions are simultaneously entering the gut from the blood. The total turnover of sodium between the intestine and blood in the course of 83 minutes is equal to the total plasma sodium.[108]

[105] G. Sörensen and K. Zerahn, *personal communication.*

[106] W. T. Pommerenke and P. F. Hahn, *Proc. Soc. Exptl. Biol. Med.,* **52,** 223 (1943).

[107] M. L. Montgomery, G. E. Sheline, and I. L. Chaikoff, *Am. J. Physiol.,* **131,** 578 (1940/41).

[108] M. B. Visscher, R. H. Varco, C. W. Carr, R. B. Dean, and D. Erickson, *Am. J. Physiol.,* **141,** 488 (1944).

Adrenalectomized rats fed a standard diet with tap water show an increased rate of excretion of administered radiosodium and a diminished rate of excretion of radiopotassium. The administration of adrenal cortical hormone to normal rats causes a retention of radiosodium and an increased excretion of radiopotassium.[109]

Labeled sodium was also employed to test the enteric coatings of capsules. Radioactive sodium chloride was placed in the capsules, using two counters simultaneously, one to measure the activity in the hand as indicator for the appearance of salts in the body and one to follow the capsule containing the salt on its way through the digestive tract. One can thus detect whether the capsule dissolves in the stomach, develops a leak, letting the salt slowly escape, or whether it breaks in the small intestines.[110]

Cope and associates[111] investigated whether and to what extent radiosodium is absorbed from the stomach of the dog. They found 1–7% of the sodium introduced into a pouch of the stomach body area to be absorbed in 1 hour. From 1 to 50% of the sodium introduced into the antral pouch was absorbed during the same period. Sodium was secreted with the gastric juice. Tangible amounts of ^{24}Na placed in a stomach pouch were found in the blood serum after 15 minutes. The fluid within a pouch of the stomach is the resultant of secretion and reabsorption, thus representing the contents and not the secretion. Two or three times as much sodium was absorbed when the acid-secreting mucosa of the stomach was in the resting than when the tissue was in the secreting state. Whether the stomach was fasting or secreting made less difference to absorption from the antrum than to absorption from the body. The antrum, it is concluded, plays a preponderant part in absorption from the whole stomach.

The absorption of heavy water was investigated by means of a similar method.[112] Half the water within the pouch was found to cross the membrane each 20 minutes.

The excretory cycle in *Tenebrio molitor* L. larvae was studied using radiosodium as a tracer. The data obtained established the fact that there is a definite circulation of sodium ion, which is absorbed by the tubules, transmitted by way of the digestive tract, and reabsorbed by

[109] E. Anderson, M. Joseph, and H. M. Evans, *J. Applied Phys.*, **12**, 317 (1941).

[110] K. Lark-Horowitz, *J. Applied Phys.*, **12**, 317 (1941).

[111] O. Cope, W. E. Cohn, and A. G. Brenizer, Jr., *J. Clin. Invest.*, **22**, 103 (1943).

[112] M. B. Visscher, E. S. Fetcher, C. W. Carr, H. P. Gregor, M. S. Bushey, and D. E. Barker, *Am. J. Physiol.*, **142**, 550 (1940).

the rectum. Furthermore, radiosodium was used to demonstrate the path of excreted material in the yellow meal worm.[113]

XI. Potassium

Ten minutes after administration of 5 mg. labeled potassium chloride to fasting rats the stomach contents were found to contain 43% of the administered potassium and the small intestine 9%, 48% having been removed. About 90% of the potassium was taken up within 30 minutes. The absorption of potassium appears to be considerably slower than that found for sodium. The urinary secretion of potassium proceeded at a nearly constant rate of about 6 to 7% per day during the 66 hours over which it was followed. The excretion of labeled potassium by the rat was found to proceed at the same rate, regardless of whether it was given orally or by intraperitoneal injection.[114] In the course of 3.5 hours, 1.7% of the labeled potassium administered by intravenous injection was excreted by the kidneys of the rabbit.[115] Of subcutaneously injected potassium, 14.4% was excreted in 48 hours, 13.4% through the kidneys, and 1.0% through the bowels.

TABLE 44

Excretion of Orally Administered Labeled Potassium[103]

Time	^{42}K recovered in urine,[a] per cent of administered
12 min.	0.0053
30 min.	0.048
3 hr.	0.46
5.25 hr.	0.65
16.25 hr.	4.75
40.50 hr.	3.00
48.25 hr.	1.56
Total	10.47

[a] Volume of urine = 2.041 ml.

An increased urinary excretion of sodium and a urinary retention of potassium are detectable 24 hours after adrenalectomy.[116]

[113] R. L. Patton and R. Craig, *J. Exptl. Zool.*, **81**, 437 (1939).

[114] M. Joseph, W. E. Cohn, and D. M. Greenberg, *J. Biol. Chem.*, **128**, 673 (1939).

[115] L. Hahn, G. Hevesy, and O. Rebbe, *Biochem. J.*, **33**, 1549 (1939).

[116] E. Anderson, M. Joseph, and V. Herring, *Proc. Soc. Exptl. Biol. Med.*, **42**, 782 (1939).

A human subject on a normal diet was found to excrete through the kidneys, in the course of 2 days, 10.5% of the labeled potassium administered (see Table 44) and, through the bowels, 0.15%.[103]

As was to be expected, the specific activities of plasma potassium, urine potassium, and saliva potassium were found by Fenn and co-workers[117] to be equal, the relative figures obtained being 1.79, 1.79, and 1.77 respectively.

The distribution of labeled sodium and potassium in different organs is discussed on pages 205 and 222.

XII. Calcium and Strontium

In the study of the calcium metabolism, ^{45}Ca is used as an indicator. This isotope has a half-life of 180 days. The radiation emitted by ^{45}Ca is so soft that counters with very thin windows must be used (see page 47).

The ^{45}Ca excreted by a rat weighing 259 g., after the administration of 250 mg. calcium lactate, is shown in Figure 33. The amount appearing in the feces is 10.8%; it is composed partly of unabsorbed ^{45}Ca and partly of ^{45}Ca secreted from the body proper into the intestines. At least 89.2% of the labeled calcium administered was thus absorbed from the alimentary tract. The unexcreted ^{45}Ca (23.6% of that administered) 69 hours after the start of the experiment was found by Campbell and Greenberg[118] to have the distribution shown in Table 45. The skeleton contains 89.5% of the total ^{45}Ca present in the rat.
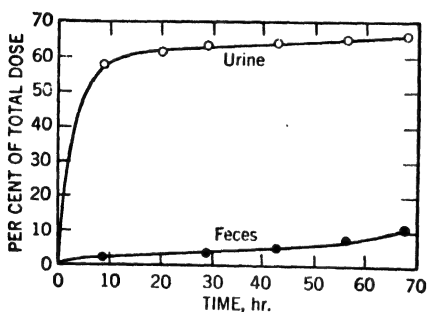
Fig. 33. Excretion by rat of radio-calcium administered as lactate.[118]

The uptake of radiocalcium by mice was found by Pecher[119] to be greater than the uptake of radiostrontium; however, the distribution among the different tissues is almost the same for the two elements, as

[117] W. O. Fenn, T. R. Noonan, L. J. Mullins, and L. Haege, *Am. J. Physiol.*, **135,** 149 (1941/42).

[118] W. W Campbell and D. M. Greenberg, *Proc. Natl. Acad. Sci. U. S.*, **26,** 176 (1940).

[119] C. Pecher, *Proc. Soc. Exptl. Biol. Med.*, **46,** 86, (1941).

becomes obvious from Table 46. Adult mice were killed 24 hours after administration of ^{45}Ca and ^{89}Sr, respectively, given as lactate by intravenous injection.

TABLE 45

Distribution of Labeled Calcium in the Rat[118]

Tissue	^{45}Ca per g. fresh weight	^{45}Ca in whole tissue, per cent of administered
Bones.....................		19.9
Teeth.....................		1.13
Serum....................	0.007	
Muscle....................		0.76
Skin and hair.............	0.028	1.43
Stomach..................	0.004	0.008
Small intestine...........	0.017	0.099
Large intestine...........	0.022	0.037
Liver.....................	0.005	0.049
Kidneys..................	0.003	0.007
Spleen...................	0.010	0.008
Heart....................	0.020	0.017
Lungs....................	0.040	0.018
Testes...................	0.006	0.031

The observation that the uptake in the skeleton is about three times higher after intravenous than after oral administration suggests that approximately one-third the ingested dose of calcium or strontium salt (chloride, lactate, or gluconate) is absorbed by the intestine.

TABLE 46

Distribution of Administered ^{45}Ca, ^{89}Sr, and ^{32}P in Organs of the Mouse after Twenty-Four Hours[119]

	Per cent administered dose per gram wet weight					
Element	Bone	Muscle	Skin and brain	Digestive tract	Liver	Other viscera
^{45}Ca	22	0.33	0.20	0.36	0.12	0.23
^{89}Sr	12	0.17	0.15	0.23	0.07	0.13
^{32}P	5.2	1.4	0.75	1.3	3.0	2.1

During the first day after subcutaneous administration of 2.3 mg. labeled strontium to rats, 17% of the dose was excreted in the urine and 6% in the feces. On the eighth day, both urine and feces contained

about 0.3%. A few days after injection of radiocalcium or radiostrontium to pregnant rats, the specific activity of the whole body of the offspring was frequently higher than the specific activity of the mother's bone. This is mainly due to the fact that calcium is incorporated into the growing body at a remarkable rate, while the uptake of labeled calcium into the deeper layers of the skeleton apatite of the mother is a very slow process.

Fig. 34. Radioautographs showing distribution of radiophosphorus (left) and radiostrontium (right) in rats.[119]

Radiostrontium shows a marked retention in the kidneys of rats injected with parathyroid hormone.[120]

Rachitic animals excrete 100% of the radiostrontium administered,

[120] W. R. Tweedy, *Federation Proc.*, **3**, 63 (1944); *J. Biol. Chem.*, **161**, 105 (1945).

while but 60% is excreted by normal animals. The excretion figures are influenced by the administration of vitamin D. It is suggested by Weissberger[121] that radiostrontium be used as an indicator of the antirachitic action of vitamin D.

In Figure 34 a comparison between the distribution of strontium and phosphorus is shown by the use of the technique of radioautography. In this experiment, carried out by Pecher,[119] one mouse received radiophosphorus, and the other was given radiostrontium. After 2.5 days, the animals were killed and sections of the entire animals were prepared and placed upon films in order to obtain the radioautographs. It will be seen in the figure that radiophosphorus accumulated in the skeleton as well as throughout the soft tissues, while almost all the radiostrontium assimilated by the animal was in the bone structures.

TABLE 46A

Radiostrontium Distribution in Organs of a Woman Three Days after Administration of 0.3 Millicurie[122]

Organ	$^{89}Sr, \mu c$ per g. wet wt., $\times 1000$	Organ	$^{89}Sr, \mu c$ per g. wet wt., $\times 1000$
Fat	0.18	Stomach	1.03
Liver	0.58	Endocardium	1.33
Brain	0.67	Small intestine	1.33
Heart	0.69	Large intestine	3.12
Spleen	0.73	Aorta	3.39
Breast tumor	0.75	Sternal cartilage	29
Liver metastasis	0.77	Whole rib	57
Muscle	0.78	Bone (femur head)	140
Lung	0.90	Bone (femur head)	159
Kidney	0.99	Bone (femur shaft)	209

Since radiostrontium of great activity, having a convenient half-life (55 days) and emitting fairly penetrating β-rays, can easily be prepared, and since this radioelement is selectively deposited in the bones, it may prove useful in clinical therapy. When radiostrontium was administered for 3 days to a female patient dying from generalized carcinomatosis, Lawrence[122] found an extensive uptake by the skeleton; the uptake in the soft tissues was almost negligible, as seen in Table 46A.

The secretion of strontium in the milk was studied by injecting

121 L. H. Weissberger and P. L. Harris, *J. Biol. Chem.*, **144**, 287 (1942).
122 J. H. Lawrence, *Am. J. Roentgenol. Radium Therapy*, **48**, 283 (1942).

labeled strontium lactate solution intravenously into lactating mice; after 2 days, 17% was recovered in the offspring.[119] Each of two Holstein cows, both in the fourth month of their lactation period, received 850 mg. labeled strontium as strontium lactate, which emitted 0.5 curie of β-radiation; 3.9% and 2.3%, respectively, were secreted in the milk of the cows during 0.6 hour following its administration, the corresponding figures during 4.5 days being 7.9% and 11%, respectively.[123]

XIII. Radium

In the study of the distribution of radium, its isotope, thorium X, was often employed as a tracer. Since the activity of 1 μg. thorium X is over 10^5 times larger than the activity of 1 μg. radium, and, since thorium X is much less costly than radium, the first-named isotope can

TABLE 47

Thorium X Content of Organs of the Rat at Various Times after Subcutaneous Injection[124]

Organ	Activity × 10 per g. fresh tissue, per cent of injected after				
	3 hr.	24	72	96	144
Blood	0.97	0.21	0.04		
Lungs	1.16	0.28	0		
Liver	0.42	0.17	0		
Kidneys	7.34	2.87	0.71	0.87	0
Testes	0.62	0.3	0		
Adrenal	1.72	1.06	0		
Intestinal wall	3.38	0.63	0.08		
Intestinal contents	14.1	4.72	2.44		
Skeleton	16.7	40.0	66.2	111.0	33.0
Bone marrow	6.91	1.5	0		0
Thyroid	16.3	12.6		3.82	0

be advantageously applied in tracer work. The distribution of thorium X in various organs, determined by Wolf and associates,[124] is shown in Table 47. It accumulates to a large extent in the skeleton and shows a behavior similar to its homologue calcium. A remarkably high uptake takes place in the thyroid gland. The appearance of an appreciable

[123] L. A. Erf and C. Pecher, *Proc. Soc. Exptl. Biol. Med.*, **45**, 762 (1940).

[124] P. M. Wolf, H. J. Born, and A. Catsch, *Strahlentherapie*, **73**, 509 (1943).

percentage of administered thorium X in the intestine had already been observed in early experiments.[125]

The accumulation of radium in the skeleton is also shown by radio-autographs of organs of the mouse or the rat after administration of 0.008 mg. and 0.05 mg., respectively.[126] Radium accumulates, in decreasing order, in the liver, kidneys, lungs, and spleen. In the muscles, heart, brain, and testes, only traces are found.[127] Of radium given by intravenous injection, 2% was found present after the lapse of 1 year.[128]

Part (about 45% in chronic cases) of the total amount of radium stored in the skeleton and tissues of a victim of radium poisoning gives rise to radon (radium emanation) in the expired breath. The radon, and consequently the amount of radium producing it, is determined by means of electrical tests in the expired air. The remainder of the body radium is determined by the γ-rays from its decay product, radium C. Evans[129] also measured the rate of loss of radium by the patients directly in these experiments by radium analyses of the feces and the urine. For chronic cases, 0.005% per day was found to be eliminated, 91% through the bowels and 9% through the kidney.

XIV. Lead

The distribution of lead in the organs has often been investigated with the aid of radium D[130,131] and thorium B[124,132-134] as indicators. In Table 48 figures are given for the distribution of thorium B in the organs of the rat. Figure 35 shows a radioautograph by Lomholt of sections of organs of a rat, secured 24 hours after administration of 0.2 mg. labeled lead chloride.[131] It is clearly shown by the figure that the organ most active in taking up lead is the kidney.

[125] O. Brill, A. Kriser, and L. Zehner, *Strahlentherapie*, **1**, 347 (1912). T. Plesch, L. Karczag, and B. Keetman, *Z. exptl. Path. Therap.*, **12**, 1 (1913). P. M. Wolf, and H. J. Born, *Strahlentherapie*, **70**, 342 (1941).

[126] A. O. Gettler and C. Norris, *J. Am. Med. Assoc.*, **100**, 400 (1933).

[127] F. Daels, H. Fajerman, and Van de Putte-Van Hove, *Strahlentherapie*, **63**, 545 (1938).

[128] B. Rajewsky, *Strahlentherapie*, **56**, 703 (1936).

[129] R. D. Evans, *Am. J. Roentgenol. Radium Therapy*, **37**, 368 (1937).

[130] J. A. Christiansen, G. Hevesy, and S. Lomholt, *Compt. rend.*, **179**, 241 (1924).

[131] S. Lomholt, *J. Pharmacol.*, **40**, 235 (1930).

[132] B. Behrens, *Arch. exptl. Path. Pharmacol.*, **109**, 332 (1925).

[133] S. Lomholt, *Biochem. J.*, **18**, 693 (1924).

[134] G. Hevesy and O. Wagner, *Arch. exptl. Path. Pharmacol.*, **149**, 336 (1930).

After subcutaneous injection of 10 mg. lead as lead nitrate (PbNO$_3$) containing thorium B, the activities of the organs of the rat were found to be only 0.1 or 0.01 of the activities measured after the injection of
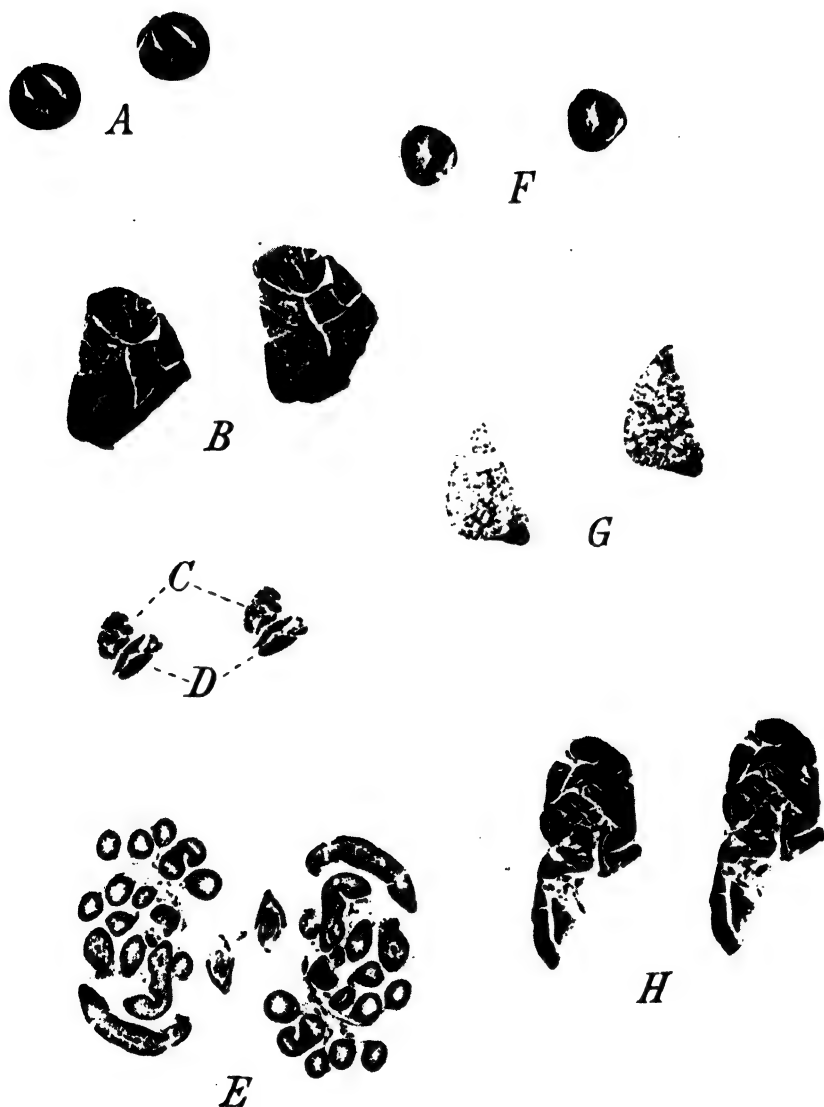
Fig. 35.   Radioautograph[131] of sections of organs from a 10-g. rat injected subcutaneously with radioactive lead chloride: *A*, kidneys; *B*, liver; *C*, pancreas; *D*, spleen; *E*, intestines; *F*, heart; *G*, lungs; *H*, brain. The photographic action is due mainly to α-rays emitted by RaF produced by RaD.

thorium B alone. No difference was found, however, when the mode of administration was intravenous injection. These results indicate that the presence of comparatively large amounts of lead greatly diminishes the percentage absorption of the subcutaneously injected labeled lead and also that the percentage distribution of lead is independent or almost independent of the amount of lead present in the circulation. The presence of thorium B produced by the disintegration of accumulated thorium X in an organ could be ascertained in other organs.[124]

TABLE 48

Distribution of Thorium B in Organs of the Rat at Various Times
after Subcutaneous Injection[124]

Organ	Activity × 10 per g. fresh wt., per cent of injected after				
	1 hr.	3	6	24	72
Blood.................	5.66	13.4	9.44		0.27
Lungs................	3.03	4.58	3.4	2.31	0.55
Liver.................	5.5	5.5	9.69	5.59	1.39
Kidneys..	16.2	16.2	88.1	47.1	5.25
Testes................	0.55	0.55	2.56	1.35	
Adrenal...............	2.16	2.16	4.16	3.34	0
Intestinal wall...........	1.76	1.75	2.71	2.0	0.35
Intestinal contents.......	4.35	4.35	2.77	2.16	
Bones................	6.82	6.82	19.4	4.34	11.2
Bone marrow...........	3.38	3.38	7.33	6.55	0.71
Thyroid...............	2.18	2.18	3.83	4.47	0.79

The percentage of lead eliminated in the course of 17 days after injection of labeled lead into the muscles of the rat amounts to 50%, two-thirds being eliminated through the bowels.[130,133]

One gram of tumor tissue takes up about the same amounts of labeled lead as one gram of muscle or viscera tissue.[134,135]

By making use of radium D as an indicator, pectin was found to inhibit lead absorption to some extent. While control rats retained 15.8% of the lead added to the diet (0.04 p.p.m.), pectin-fed rats retained 11.8% only.[136]

Rats with their bodies immersed in water to prevent absorption through the skin, were exposed to the vapor of radioactive tetraethyl-

[135] C. Ehrenberg, Z. Krebsforsch., **35**, 348 (1932). C. Dittmar, ibid., **48**, 121 (1938/39).

[136] H. K. Murer and L. A. Crandall, Jr., J. Nutrition, **23**, 249 (1942).

lead. The amount of lead absorbed in a given time was nearly proportional to the concentration of the vapor, and it was calculated that 16 to 23% of the vapor reaching the alveoli entered the blood.[137] The presence of gasoline vapor does not affect the absorption rate of tetraethyllead. An uptake of 11.4 mg. per kilogram body weight was found to be fatal, the compound being rapidly destroyed in the body.[137]

XV. Antimony

Radioactive antimony, administered as stibine gas (SbH_3) to chicks, both normal and infected with *Plasmodium gallinaceum*, and to normal guinea pigs, has been measured in the blood and tissues at successive time intervals following its administration.[137a] Significant differences were not apparent between distributions in normal and infected groups.

The concentration of antimony in the blood stream exhibited a smoothly decaying curve, decreasing more rapidly in the guinea pig than in the chick. The red corpuscles contained a much higher concentration of antimony than did the plasma. The concentration curves of antimony in lung, brain, muscle, and fat were generally similar to that of blood, while those of the liver, and to a lesser extent the spleen, passed through a maximum about 1 hour following treatment. Concentration curves of the kidney and heart were of variable shape. Approximately 4 hours after treatment the tissue antimony levels became constant with respect to order of rank; liver, spleen, and kidney were greater than whole blood; all other tissues showed less than the blood but as much or more than the plasma.

Antimony administered as stibine is excreted, apparently in the trivalent state, via the bile and urine in the guinea pig. The rate of elimination from the body is shown to be much higher for the guinea pig than for the chick.

It was found by Brady et al.[137b] that when a single therapeutic dose of antimony was administered to dogs either as tartar emetic or sodium antimonyl xylitol, the thyroid at the end of 36 hours contained a higher concentration of antimony than did any other organ of the body except the liver. Moreover, 36 hours after completion of 12 injections of

[137] R. A. Mortensen, *J. Ind. Hyg. Toxicol.*, **24**, 258 (1942).
[137a] Radiobiology group at the Naval Medical Research Institute and Department of Terrestrial Magnetism, *in press*.
[137b] F. J. Brady, A. H. Lawton, D. B. Cowie, H. L. Andrews, A. T. Ness, and G. E. Ogden, *Am. J. Trop. Med.*, **25**, 103 (1945).

sodium antimonyl xylitol[137c] given over fourteen days, the concentration of antimony in the thyroid exceeded that in the liver. A high accumulation of antimony was also observed in the adult filarial parasites (*Dirofilaria immitis*), which ranked third highest in antimony concentration in the infected dog.

XVI. Bismuth

Christiansen and co-workers[138] found that bismuth shows a behavior very different from that of lead. The urine contained 66–75% of the labeled bismuth excreted, and only 25–33% was found in the feces.

The distribution of labeled bismuth in the guinea pig 10 days after subcutaneous injection of 8 mg. bismuth as a solution of bismuth oxychloride in water was determined by Lomholt.[139] The results are shown in Table 48A.

TABLE 48A

Distribution of Labeled Bismuth in a Guinea Pig[139]

Organ	Bi distribution per 100 g. tissue, per cent of administered dose[a]	Organ	Bi distribution per 100 g. tissue, per cent of administered dose[a]
Heart........	0.031	Brain.........	0.018
Lungs........	0.152	Blood serum...	0.020
Liver.........	0.161	Skeleton......	0.044
Spleen........	0.213	Muscles.......	0.013
Kidneys......	2.881	Skin..........	0.034

[a] Dose = 8 mg. Bi as aqueous BiOCl. Unabsorbed Bi = 50.1% of amount injected. Bi excreted = 42.9% of amount injected.

Labeled bismuth was used in the study of the rate of absorption of various bismuth compounds employed in syphilis therapy.[139]

XVII. Polonium

Polonium is chiefly taken up by the cells constituting the reticuloendothelial system. Lacassagne[140] administered polonium and carmine red simultaneously to rabbits and found that the cells taking up the

[137c] D. B. Cowie, A. H. Lawton, A. T. Ness, F. J. Brady, and G. E. Ogden, *J. Wash. Acad. Sci.*, **35**, 192 (1945).

[138] J. A. Christiansen, G. Hevesy, and S. Lomholt, *Compt. rend.*, **178**, 1324 (1924).

[139] S. Lomholt, *Handb. d. Haut- und Geschlechtskrankheiten.* Springer, Berlin, 1928.

[140] A. Lacassagne, J. Lattes, and L. Lavedan, *J. Radiologie*, **9**, 1 (1925).

Fig. 36.   Radioautograph of organs of rabbits[140] injected with polonium: *1* and *2*, kidney; *4*, heart; *5*, placenta; *6*, liver; *7*, brain; *8*, lung; *9*, pancreas; *10* and *14*, lymphatic ganglia; *11*, ovary and testicle; *12*, suprarenal capsule; *13*, bone marrow; *15*, intestine; *16*, spleen; *17*, appendix; *18*, bone; *19*, skin; *20*, thymus.

carmine red seem to be those which contain the most polonium. An especially marked uptake was found in the endothelial cells of the spleen, in the lymphoid formations of the lungs, in the cortical part of the thymus, in the perivascular and endothelial part of the bone, the marrow, etc. The uptake of carmine red was followed by the usual microscopic technique, and the uptake of polonium was tested by taking microradiophotographs of the tissue slices. Polonium emits α-rays, which act on the photographic plate producing a fairly sharp picture of the tissue slice containing a sufficient amount of polonium. The distribution of polonium between the different organs of the rabbit is seen in the striking radioautograph taken by Lacassagne (Figure 36). Lacassagne has also demonstrated pathological changes in the kidney by taking such radioautographs.

XVIII. Thorium

The distribution of thorium in normal and in tumor mice was investigated by using ionium as an indicator.[134] In the course of 9 days, 99.1% of the 0.25 mg. labeled thorium administered by subcutaneous injection was excreted; of the remaining thorium, the liver contained 16.0%, the kidneys 10.2%, and the spleen 15.6%.

In experiments of only 4 hours' duration, using uranium X as an indicator, the greatest percentage of thorium administered by intravenous injection was found in the spleen, the percentage uranium X content decreasing in the order: liver, marrow, and kidney. The thorium content of the blood is reduced to 20% of its initial value in the course of 4 hours.[141] After injecting colloidal thorium dioxide intravenously into rabbits, more than 99% of the thorium was found to be removed from the blood stream within 6 or 8 hours.[142]

XIX. Plutonium

As stated by Stone[143] humans excrete in the urine between 0.005 and 0.01% per day of the amount of plutonium deposited in the body. The maximum permissible dose in the human has been estimated to be 1 μg. From the facts accumulated regarding the lethal effects of fission products in the human body, it can be estimated that man would be

[141] P. M. Wolf, Gh. Radu, A. Carsch, and H. J. Born, *Strahlentherapie*, **75**, 452 (1944).

[142] F. A. Maxfield and O. A. Mortensen, *J. Applied Phys.*, **12**, 197 (1941).

[143] R. S. Stone, *Proc. Am. Phil. Soc.*, **90**, 11 (1946).

killed by about 10 microcuries per gram body weight. Plutonium has been found to locate in the liver; its incorporation into the bones is discussed in Chapter 10.

The first day following intravenous injection of 40 to 400 μg. $PuO_2(NO_3)_2$ to rats the urinary excretion of plutonium was 7.5% of the dose as compared with 0.33, 0.57, and 0.71% when administered as $PuCl_3$, $Pu(NO_3)_4$, and Pu^{4+} citrate complex, respectively. Fecal excretion during the first day was correspondingly lower following injection of $PuO_2(NO_3)_2$. On the thirtieth day following intravenous injection there were no significant differences in either urinary or fecal excretion of plutonium administered as $PuCl_3$, $Pu(NO_3)_4$, Pu^{4+} citrate complex, and $PuO_2(NO_3)_2$. At this time the average urinary excretion was 0.014% of the injected dose and the average fecal excretion was 0.22%. The average ratio of fecal to urinary excretion was 16 : 1.

The skeleton was a major site of deposit regardless of the form in which the plutonium was injected. Four days following injection of plutonium as $PuCl_3$, $Pu(NO_3)_4$, Pu^{4+} citrate complex, and $PuO_2(NO_3)_2$, skeletal deposition was 44.9, 29.3, 56.9, and 56.5% of the injected dose, respectively. Deposition in the liver under the above conditions was 22.9, 39.7, 9.6 and 9.1% of the injected dose, respectively. Deposition of plutonium in the kidney and spleen and in general was not greatly affected by the form in which the plutonium was administered.

The size of the injected dose of Pu^{4+} citrate complex did not affect the percentage of the dose excreted in the feces and urine. Likewise, the size of dose did not alter the percentage of injected material present in the various tissues 6 days following injection.

Absorption of plutonium from the gastrointestinal tract was quite low — 0.01% of the administered dose. When Pu^{4+} was administered orally in 5% sodium citrate solution, the presence of the citrate seemed to increase absorption of the plutonium from the gastrointestinal tract. Absorption was still quite low — only 0.3% of the administered dose.

Of the plutonium absorbed from the gastrointestinal tract 79% was deposited in the skeleton and 7.3% in the liver (see Table 49). Plutonium absorbed slowly via this route seems to give a higher deposition in the skeleton than it does when administered intravenously as $PuCl_3$, $Pu(NO_3)_4$, Pu^{4+} citrate complex, or $PuO_2(NO_3)_2$.[143a]

The excretion of plutonium is promoted by intravenous injection of zirconium citrate. The zirconium first acts to displace plutonium from

[143a] W. Langham, *in press*.

the liver.   Later the zirconium migrates to the bone and slowly but continuously displaces the deposited plutonium to an extent which depends on the concentration of zirconium relative to the plutonium.

TABLE 49

Absorption and Body Distribution of Plutonium
Administered Orally to the Rat[143a]

Total dose Pu[a]	Concentration of $Na_3C_6H_5O_7 \cdot 5\frac{1}{2}H_2O$	Dose absorbed, %	Absorbed Pu per organ, %		
			Liver	Skeleton	Balance
46	5	0.34	7.8	70.8	21.4
198	5	0.32	8.2	81.7	10.1
488	5	0.25	3.7	88.1	8.2
488	0	0.01	7.5	80.0	12.5
2000	0.5	0.06	9.4	74.2	16.3
		Average:	7.3	78.9	13.8

[a] All doses were administered by giving one-fifth of the total amount of plutonium per day for five consecutive days.

Within 24 hours after injection of zirconium in sodium citrate solution, 80–90% of the metal was excreted in the urine.   Fecal excretion of zirconium is very low.[143b]

XX. Iron

A. DISTRIBUTION IN TISSUES OF DOGS

In general, the body controls its stores of various substances by excretion and by catabolic processes.   The use of labeled iron in the study of the uptake of iron by the dog by Hahn and his colleagues[144] has shown conclusively that the body controls its iron stores by absorption or lack of absorption rather than by its capacity to eliminate iron.

A dog, *a*, weighing 4.5 kg., was placed on a diet low in iron, and consisting chiefly of white bread and canned salmon.   It was made anemic by bleeding, and anemia was maintained for 3 weeks.   Radioactive iron was fed in the form of $Fe_2(SO_4)_3$ at a level of about 55 mg. daily (with a relative activity of 100 units) for 4 days.   Twenty hours after the last feeding the animal was subjected to viviperfusion in order to free

[143b] T. Schubert, *in press*.

[144] P. F. Hahn, W. F. Bale, E. O. Lawrence, and G. H. Whipple, *J. Exptl. Med.*, **69**, 739 (1939).   P. F. Hahn, W. F. Bale, R. A. Hettig, M. D. Kamen, and G. H. Whipple, *ibid.*, **70**, 443 (1939).

the viscera of blood.   The final hematocrit value at the end of this
procedure was 0.4%, in contrast to 17% at the beginning.   The anemia
of dog b, weighing 4.2 kg., was maintained for 9 weeks in order to
remove all iron reserves.   In the case of dog c, weighing 6 kg., anemia
was continued for 5 weeks.   Some of the results obtained are shown in
Table 49A.   The average radioiron content of the anemic dogs works
out to be 58 times that of the normal dog.

TABLE 49A

Radioactive Iron Content of Tissues[144]

Dog	Anemic			Normal	
	a	b	c	a	b
Number of feedings	4	2	1	18	5
Iron fed (mg.) counts per min	220	66	130	650	103
Relative activity counts per min.	770	464	5730	600	2120
Hemoglobin level, per cent when fed	39	62	53	178	138
Hours after last feeding	20	20	23	84	23
Organ	Radioiron found, per cent of total amount fed				
Liver	0.4	0.4	0.5	0.2	0.03
Spleen	0.0	0.0	0.1	0.0	0.02
Marrow	0.2	3.0	2.0	0.0	0.03
Plasma	0.0	0.3	0.1	0.0	0.01
Red corpuscles	8.7	9.0	1.4	0.04	0.06
Total	9.3	12.7	4.1	0.24	0.15

In another set of experiments, the radioactive iron was administered
by intravenous injection as ferrous gluconate, and the excretion of the
radioiron in urine, bile, and feces was determined.   It is generally
accepted that the urine is not a factor in iron loss from the body and the
application of the radioactive indicator has shown this to be true,
except for a short period of iron excretion following an intravenous dose
of iron (see Table 50) when the radioiron concentration of the plasma
is very high.   Similarly, the first period following the injection shows
an appreciable excretion of iron in the feces, but in subsequent periods
the amount is considerably diminished.

The output of radioiron in the feces during long periods (excepting
1 or 2 weeks following administration by vein) is quite uniform.   The
daily output runs between 0.05 and 0.40 mg.; large dogs eliminate some-
what more.   It is significant that the plethoric dog eliminates no more

than does the anemic dog where conservation of iron must be active.[144]

Considerable variation in percentage absorption of single doses of radioiron takes place. To explain this fact, the suggestion was made that the absorption of iron even in an anemic dog with an empty gastro-intestinal tract is never 100%; rather, 50% is maximum for small doses and 5% for large doses (400 mg.). Iron absorption takes place in the stomach and the small intestine, perhaps more taking place in the

TABLE 50

Excretion of Intravenously Administered Labeled Iron[a]
by an Iron-Depleted Dog[144]

Source	Days after injection	Iron excreted, per cent of injection
Urine	2	3.7
	2	0.015
	5	0.011
	6	0.008
Feces	9	1.90
	6	0.50
	7	0.60

[a] 6.5-kg. dog, depleted by 2-month continuous anemia due to bleeding, given 105 mg. labeled iron.

stomach and the duodenum. Contact duration of the solution of iron with the mucosa is important, and many variables enter here to account for lack of uniformity of absorption — time of stay in stomach, for example, may vary greatly due to mechanical irritation or emotion. The formation of hemoglobin in the anemic dog takes place at a very rapid rate. When red corpuscle production has been accelerated by iron feeding or diet factors, or when the dose of iron is very small, the dog can turn out almost all the absorbed radioiron as hemoglobin within 2 or 3 days; this is discussed in greater detail on page 492. Radioiron is detectable in the circulating red corpuscles 4 hours after feeding. The absorbed labeled iron is entirely converted into hemoglobin within 4 to 7 days under anemic conditions. In a dog with severe anemia, after exhaustion of the reserve iron, the hemoglobin produced is a measure of the iron absorbed.[144a]

Radioiron absorption from the gastrointestinal tract of the dog is impaired by the presence of a sterile abscess in the subcutaneous tissue.[144b]

[144a] P. F. Hahn, J. F. Ross, W. F. Bale, G. H. Whipple, *J. Exptl. Med.*, **71**, 731 (1940).

[144b] P. F. Hahn, W. F. Bale, and G. H. Whipple, *Proc. Soc. Exptl. Biol. Med.*, **61**, 405 (1946).

This finding is of interest because of the frequent occurrence of anemias associated with infections and inflammatory disease.

B. DISTRIBUTION IN TISSUES OF RATS AND RABBITS

The absorption, distribution, and excretion of iron were also studied in normal and iron-deficient rats.[145] Radioiron was administered as ferric chloride solution by stomach tube in doses of 2–8 mg. to rats. During the 10-day period, the normal animals (hemoglobin values ranging from 14.4 to 16.1 g. per 100 ml.) retained about 30% of the iron administered, while the anemic animals (hemoglobin values ranging from 5.9 to 9.3 g. per 100 ml.) retained 50%.

After administering 0.7 mg. iron parenterally to rats, only a small percentage was found to be excreted. The urine contained 1.6%, the feces 0.2%, and the bile 0.7%. In this investigation, ^{55}Fe having a half-life of 4 years was used, while in the investigations mentioned above ^{59}Fe (half-life$=47$ days) was employed.[146]

In experiments with anemic rabbits, the muscles and particularly the myocardium, showed an increased affinity for iron.[147a]

In their studies on the distribution of radioiron in organs of the rat, Copp and Greenberg[147] applied ^{55}Fe. They found the chief organ for iron storage to be the liver, with the spleen playing a secondary role and the muscle being relatively unimportant. The route of administration was found to have a profound effect on the amount of radioiron stored in the liver. The amount of ^{55}Fe in the liver was much less when the radioiron was being actively utilized in hemoglobin synthesis by bone marrow which had been stimulated by administration of cobalt and by blood loss as seen in Table 50A.

In anemic rats up to 15% of the orally administered radioiron (as normal citrate) was found in the bone marrow, that of normal rats containing somewhat less. The half-retention time of ^{55}Fe in the bone marrow was of the order of 1 or 2 days.

In two cows studied, 1.5% and 2.5% of the radioiron administered orally was secreted in the milk during a 78-hour period.[148]

[145] M. E. Austoni and D. M. Greenberg, *J. Biol. Chem.*, **134**, 27 (1940).

[146] D. M. Greenberg, D. H. Copp, and E. M. Cuthbertson, *J. Biol. Chem.*, **147**, 749 (1943).

[147] D. H. Copp and D. M. Greenberg, *J. Biol. Chem.*, **164**, 377, 389 (1946).

[147a] A. Vanotti, *Bull. Schweiz. Akad. Med. Wiss.*, **2**, 90 (1946).

[148] L. A. Erf, *Proc. Soc. Exptl. Biol. Med.*, **47**, 287 (1941).

Following intravenous administration to rabbits of bivalent and trivalent iron, changes in metabolism could be detected only during a few hours following the injection.[147a] Ferrous iron was found to be much

TABLE 50A

Effect of Route of Administration on Radioiron Content
of Blood and Liver of the Rat[147]

Condition of experimental rats	Route of radioiron administration	Radioiron in blood at 24 hr., per cent of total dose	Peak storage of radioiron in liver, per cent of total dose
Young control..................	Stomach tube ...	14	3
Young anemic................., ...	Stomach tube ...	35	4
Young control..................	Intraperitoneally.	11	39
Young anemic..................	Intraperitoneally.	33	25
Young anemic+injection of copper	Intraperitoneally.	47	7
Adult control..................	Subcutaneously ..	18	11
Adult control................., ..	Intravenously ...	15	42
Adult control..................	Intraperitoneally.	7	30
Adult given cobalt treatment.....	Intraperitoneally.	29	12
Adult following severe blood loss..	Intraperitoneally.	39	10

more readily absorbed, and subsequently utilized, than the corresponding ferric salt fed under the same conditions to humans.[149] After absorption has taken place, the iron is utilized promptly in the body and appears as hemoglobin in the red cells of the circulation within a few hours.[150] In the rabbit, iron metabolism connected with formation of the cellular hemin pigments (myoglobin and cytochrome) is found to be distinctly slower than that of hemoglobin.[147a]

C. DISTRIBUTION IN THE TISSUE OF PUPS

In pups, a considerable fraction of the absorbed iron was found by Hahn *et al.*[152] in tissues other than the circulating red corpuscles (see Tables 51 and 51A). Considerable amounts of radioiron were found in

[149] P. F. Hahn, E. Jones, R. C. Lowe, G. R. Meneely, and W. Peacock, *Am. J. Physiol.*, **143**, 191 (1945).

[150] P. F. Hahn, J. F. Ross, W. F. Bale, and G. H. Whipple, *J. Exptl. Med.*, **71**, 731 (1940).

[151] P. F. Hahn, S. Granick, W. F. Bale, and L. Michaelis, *J. Biol. Chem.*, **150**, 407 (1943). S. Granick and P. F. Hahn, *ibid.*, **155**, 661 (1944).

[152] P. F. Hahn, W. F. Bale, J. F. Ross, W. M. Balfour, and G. H. Whipple, *J. Exptl. Med.*, **78**, 169 (1943).

the spleen, which presumably acts as a depot for iron coming in through the gastrointestinal tract on its way to general body use.

TABLE 51

Recovery of Fed Radioiron from Tissues of Pups[152]

Tissue	Per cent of fed iron recovered	
	Pup 1	Pup 2
Blood and perfusate...................	6.8	6.9
Viscera..............................	1.3	1.1
Striated muscles......................	1.2	0.9
Bone marrow.........................	19	29
Total.............................	28	38

Bone marrow is found to contain an appreciable part of the fed radioiron recovered in blood and tissues, as is seen in Table 51. The radioiron (63 mg.) in this experiment was administered in the course of 27 days. These high figures indicate that the demand for new red corpuscles during growth is not as acute as in anemia and the radioiron piles up in reserve.

TABLE 51A

Distribution of Radioiron in Perfused Tissues of Growing Pups[152]

Tissue	Radioiron fed, mg. per 100 g. fresh tissue	
	Pup 1-F	Pup 2-F
Liver................................	0.202	0.183
Spleen...............................	0.430	0.397
Pancreas.............................	0.056	0.079
Heart................................	0.098	0.088
Diaphragm...........................		0.032
Foreleg..............................	0.033	0.028
Left hind leg (bound).................	0.034	0.027
Right hind leg........................	0.028	0.026
Stomach.............................	0.020	0.020
Duodenum...........................	0.031	0.028
Jejunum.............................	0.023	0.017
Ileum...............................	0.016	0.016
Colon...............................	0.017	

D. PLASMA IRON

Using the radioactive isotope of iron it is possible to distinguish between the level of iron normally present in the plasma and the increase introduced by feeding the radioiron. When frequent samples of the plasma are taken after feeding a single dose of radioiron, we find great variation in the values obtained (see Table 52). The maximum level of the plasma radioiron does not seem to be related in any way to the size of the dose or the amount absorbed as subsequently determined by the appearance of the radioiron in the red corpuscles.

However, when the iron is mixed with the food, there is a delayed rise in the plasma radioiron curve and the peak is first reached after 5 or 6 hours. Hahn and his co-workers[152] concluded from these results that there must be many variables operating to modify plasma iron curves which, therefore, introduce errors into quantitative absorption determinations calculated from such curves. The amount of radioiron of the plasma present in combination with protein was found to be about 95% of the total. Only about 15% of the iron was bound to globulin.[152a]

TABLE 52
Radioiron in Plasma of the Dog Following Feeding[152]

Dog	Iron dose, mg.	Iron salt	Time of peak, hr.	Peak level, per cent of amount fed/ 100 ml. plasma	Radioiron absorbed, %
I	64	Fe ammonium citrate...	1.3	0.13	1.4
II	55	Fe ammonium citrate...	1.0	0.36	4.2
II	58	Fe citrate............	2.0	0.70	12.2
III	36	Fe ammonium citrate...	1.5	0.19	14.0
IV	48	Fe ammonium citrate...	2.0	0.11	18.4
V	0.2	Fe citrate............	1.0	1.7	24.0
II	115	Fe chloride (mixed with food)..............	6.0	0.56	3.2
VI	42	Fe sulfate (mixed with food)..............	5.7	0.41	30.0

E. UPTAKE BY HUMAN BEINGS

When iron reserves are depleted, human beings were found to absorb iron in large amounts, in fact, 10 to 20 times normal. In order to study

[152a] H. Yoshikawa, P. F. Hahn, and W. F. Bale, *Proc. Soc. Exptl. Biol. Med.*, **49**, 285 (1942).

TABLE 53

Radioiron Uptake by Red Corpuscles in Various Clinical Conditions[153]

Diagnosis	Weight, kg.	Estimated red cell mass, ml.	Hematocrit		Interval between feeding and sampling, days	Dose iron, mg.	Red corpuscle radioiron, % of amount fed /100 ml.	Estimated circulating iron, % of amount fed
			At feeding, %	At sampling, %				
Gastric ulcer, hemorrhage	49.4	1005	18.4	33.8	11	18	1.48	15
Duodenal ulcer, hemorrhage	51.8	1455	29.4	46.7	7	20	1.37	20.2
Abortion, hemorrhage	50.0	630	22.1	20.8	4	423	0.20	1.3
Impetigo	9.8	220	35.3	37.9	7	8	0.98	2.2
Chronic pyelonephritis	52.6	600	18.4	19.0	7	42	—	0-3
Hypochromic anemia	64.6	1150	32.0	29.4	6	204	0.17	2.0
Pernicious anemia	60.0	1090	23.4	30.3	10	20	0.12	1.3
Leukemia	8.9	130	24.5	—	6	125	0.47	0.6
Familial icterus	11.8	215	—	30.5	7	93	0.13	0.3
Mediterranean anemia	14.3	46	6.6	5.3	4	15	—	0-1.5
Mediterranean anemia	13.0	175	23.2	22.7	5	64	0.26	0.5
Hemachromatosis	61.2	1610	45.6	43.8	6	53	0.04	0.7
Hemachromatosis	61.2	1610	41.0	43.4	7	14	—	0.8
Polycythemia	59.0	1750	—	49.6	6	204	0.03	0.5
Normal	81.0	2260	48.5	46.6	7	20	0.08	1.8

the amount of iron absorbed in diverse diseases, the percentage radioiron administered present in the corpuscles of patients with several diseases was determined by Balfour and colleagues.[153] A low corpuscle ^{59}Fe content was observed in the case of Mediterranean anemia and familial icterus, for example; in these diseases the iron content of the organs is high. On the other hand, a high content of ^{59}Fe in the corpuscles was found in patients suffering from gastric or duodenal ulcer (see Table 53).

The utilization of 9 to 18 mg. labeled iron administered intravenously as ascorbate was found to be prompt (up to 93%) in iron-deficient patients. Utilization of ^{59}Fe by healthy adult men was somewhat slower but almost as complete. Patients with hypoplastic anemia used less than 4% of the tagged iron. In patients with pernicious anemia, utilization was likewise slow until liver therapy was begun. In hemolytic anemias, the amounts of radioactive iron present in the peripheral blood at any one time varied widely from 10 to 90%. Dubach et al.,[153a] emphasize the importance of the observation of the prompt and almost complete utilization of the injected isotope by healthy adult men. This result is explained by assuming that newly administered iron was selectively used in preference to body iron already stored. A similar suggestion was put forward by Ross[153b] and by Greenberg and Wintrobe.[153c] Of the different fractions of storage iron, possibly ferrous and ferric compounds in the form of their hydroxides, in which the newly administered iron may have been present, represent the metabolically active iron. Because injected radioactive iron is either not completely used for hemoglobin synthesis under a variety of conditions or does not appear at all in the circulating hemoglobin, the application of the radioactive iron technique (determination of the percentage radioiron absorbed from the amount found in the red corpuscles) in the study of iron absorption from the gastrointestinal tract has its limitations. As an example, observations are presented to show that patients with untreated pernicious anemia may absorb considerable amounts of radioiron but that the isotope does not appear in the peripheral blood in

[153] W. M. Balfour, P. F. Hahn, W. F. Bale, W. T. Pommerenke, and G. H. Whipple, *J. Exptl. Med.*, **76**, 15 (1942).

[153a] R. Dubach, C. V. Moore, and V. Minnich, *J. Lab. Clin. Med.*, **31**, 1201 (1946).

[153b] J. F. Ross, *Am. Soc. Clin. Invest.*, **27**, 33 (1946).

[153c] G. R. Greenberg and M. M. Wintrobe, *J. Biol. Chem.*, **165**, 397 (1946).

large amounts until after erythrocyte maturation arrest has been relieved by administration of liver extract.

F. MECHANISM OF IRON ABSORPTION

Experiments by Hahn and colleagues,[152] which aimed at a better understanding of the iron absorption by normal and anemic dogs, gave the following results. Iron absorption is a function of the gastrointestinal mucosal epithelium. The normal, nonanemic dog absorbs little iron, but chronic anemia brings about considerable absorption, perhaps 5 to 15 times normal. Most tissues contain, over and above the indispensable, iron which can be depleted by long-continued anemia. This general depletion of iron involves the gastrointestinal tract and presumably is a factor in absorption of the metal. It is of great interest that sudden change from the normal state to severe anemia within 24 hours does not significantly increase iron absorption. As the days pass, however, new hemoglobin is formed. On the contrary, when the body iron stores are depleted, iron absorption is still active, even when the red corpuscle hematocrit is rising. Anoxemia of 50% normal oxygen concentration for 48 hours does not significantly enhance iron absorption. In this respect it resembles acute anemia. Furthermore, doses of ordinary iron given by mouth 1 to 6 hours before radioiron will cause some "mucosa block," that is, an intake of radioiron less than anticipated. Iron given by vein some days before the oral dose of radioiron does not, in contrast to the above finding, appear to inhibit iron absorption.

Balfour et al.[153] postulate the presence of a compound in the mucosa which is capable of combining loosely and reversibly with iron. Such an acceptor in the mucosa cells might be a protein, a material such as ferritin or apoferritin, which is capable of taking up iron stoichiometrically. This acceptor would be able to take up limited amounts of iron from the intestinal lumen and, in turn, to pass them on to the plasma when the iron level there was lowered. Such a mechanism would explain in part the limited ability of the body to accept iron and the relatively greater efficiency in absorption of small doses of iron. In the normal animal with a normal plasma iron, the acceptor mechanism would be physiologically saturated with iron and incapable of picking up more from the gastrointestinal tract. Desaturation requires days, not hours, whereas saturation may take place within 1 to 2 hours.

These conclusions are further supported by the recent work of

Granick[154] carried out by means of purely chemical methods. He found that, when ferrous ion is fed, ferritin increases markedly, especially in the duodenal and jejunal regions, appreciable increase being noted within 4 to 5 hours. A maximum response occurs within 7 hours after feeding. After a period of some 3 to 6 days, ferritin returns to the level of the controls.

G. FORMATION OF FERRITIN

The conversion of inorganic and hemoglobin iron into ferritin iron in the animal body was followed by Hahn and colleagues[151] by making use of radioiron as an indicator. Ferritin consists of a protein fraction, apoferritin, linked with micelles of a special type of colloidal ferric hydroxide, the crystals containing as much as 23% iron. As shown in Table 54, it was found that iron in the form of ferric ammonium citrate, when administered by vein to the dog, is converted to ferritin iron, some being taken up by liver and spleen. Substantial amounts of labeled ferritin were, as recorded in Table 55, found in the spleen in experiments in which red corpuscles containing labeled iron as a constituent of their hemoglobin were injected and the resulting mixed cells of the circulation were destroyed with acetylphenylhydrazine. A considerable part of the debris of corpuscle destruction was taken up by the liver and spleen.

TABLE 54

Ferritin Iron Formation from Intravenously Administered Ferric Ammonium Citrate[151]

Conditions and tissue	Total[a] Fe, mg.	Total activity, counts per min.	Specific activity, counts per min. per mg. Fe	Distribution of labeled Fe, per cent injected Fe	Per cent of tagged Fe found
Injected	78.0	238,000	3050	100	
Liver (323 g.)[b]	78.0	195,000	2500	82	82
F + NCF	27.3	62,000	2270	26	75
F	7.72	17,800	2310	7.5	76
Spleen (21 g.)[b]	4.95	821	166	0.35	5.4
F + NCF	1.91	466	244	0.20	8.0

[a] Ferritin (F) and noncrystallizable ferritin (NCF) fractions found in spleen and liver have been corrected to correspond to amount which would have been isolated from whole organs.
[b] Fresh weight.

It was also found that the body is able to convert injected ferric iron of the form containing five unpaired electrons to ferric iron of the form containing three unpaired electrons, characteristic of ferritin.

[154] S. Granick, *J. Biol. Chem.*, **164**, 737 (1946).

TABLE 55

Ferritin Formation from Hemoglobin Iron of Red Blood Corpuscles[151]

Conditions and tissue	Total[a] Fe, mg.	Total activity, counts per min.	Specific activity, counts /min./mg. Fe	Distribution of labeled Fe, %		Per cent tagged Fe found
				Total circulating activity, after transfusion =100%	Activity lost to circulation =100%	
Injected tagged blood (110 ml.) ...	64.2[a]	6720[b]	105[b]	100		
Circulating blood after transfusion	324	6720	20.7	100		19.8
Circulating blood before death	127	1050	8.3	15.6		7.9
Liver (335 g.)	138	3100	22.5	46.1		21.4
F + NCF	72.4	1925	26.5		55	25.3
F	12.4	332	26.8			25.5
Spleen (89 g.)	87.5	1150	13.2	17.1		12.5
F + NCF	26.1	328	12.6		20	12.0

a See footnote a, Table 54.
b Estimated.

H. TUBULAR REABSORPTION RATE

In order to test the accuracy of estimated tubular reabsorption rates in dogs, injections of hemoglobin containing radioactive iron were given to animals (Yuile and associates[155]). The actual amounts of radioiron retained by the kidneys 24 hours after injection were then determined, and the actual and estimated rates of tubular reabsorption were compared. The kidneys of normal animals were found to retain less iron than those of animals with lowered thresholds, despite the fact that the former group has a much higher estimated rate of tubular reabsorption. A lowered threshold was obtained by injecting hemoglobin repeatedly. To explain the above finding it is suggested that hemoglobin products are more rapidly removed by the kidneys of normal animals, following reabsorption, than by the kidneys of animals which have received multiple injections of hemoglobin.

TABLE 56

Partition[a] of Labeled Iron, Cobalt, and Manganese in Bile Fistula Rats[156]

Element	Mode of administration[b]	Amount, mg.	Number of animals	Bile	Urine	Feces	Gastrointestinal tract	Liver
Fe[c]	I	0.1	3	0.1 ±0.01	1.6 ± 0.5	0.2 ± 0.2	1.9 ± 1.5	
	O	0.1	2	0.2 ±0.1	1.4 ± 0.9	28.5 ±10.2	20.6 ±3.3	
Co[c]	I	0.1	5	3.5 ±1.4	63.5 ± 8.5	4.9 ± 0.6		2.5 ±0.6
	O	0.1	2	2.0 ±0.2	18.5 ±12.0	39.6 ±12.0		3.5 ±0.7
Mn[d]	I	0.01	2	27.1 ±0.4	5.4 ± 1.4	5.6 ± 0.9	21.2 ±0.9	27.1 ±3.5
		0.1	3	37.3 ±8.6	3.2 ± 2.1	6.5 ± 1.7	7.3 ±1.0·	11.7 ±3.7
	O	0.1	2	1.1 ±1.0	1.2	39.2 ± 2.3	42.7 ±4.4	

[a] Per cent of administered dose·
[b] By injection (I) or orally (O).
[c] 72 hours after administration.
[d] 48 hours after administration.

XXI. Cobalt and Manganese

Comparison of the elimination of manganese, cobalt, and iron in the bile demonstrates the already discussed (see page 167) limited ability of the mammalian organism to eliminate iron incorporated in the body. By making use of ^{56}Mn, of a mixture of ^{56}Co and ^{58}Co, and of ^{55}Fe as

[155] C. L. Yuile, J. F. Steinman, P. F. Hahn, and W. F. Clark, *J. Exptl. Med.*, 74, 197 (1941).

tracers, the excretion of manganese, cobalt, and iron by the dog was compared by Greenberg et al.[156] An artificial gall bladder type of fistula was employed. Very substantial amounts of manganese are secreted into the bile, as is shown in Figure 37. Manganese is excreted almost
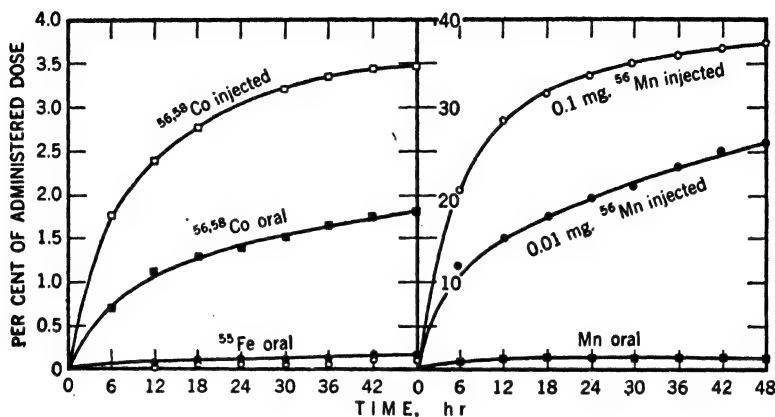
Fig. 37. Rate of elimination of radioiron, radiocobalt, and radiomanganese in the bile.[156]

entirely with the feces (see Table 56), the bile playing an important part in the intestinal excretion. The urine is the chief pathway for the excretion of cobalt. Orally administered cobalt is only partially absorbed and manganese very poorly.

In view of the importance of cobalt deficiency diseases in the livestock industry detailed investigations were carried out on the excretion and tissue distribution of radiocobalt.[156a] The radiocobalt employed was obtained by bombardment of iron with deuterons and consisted of a mixture of 3 cobalt isotopes with half-lives ranging from 65 to 270 days. The cobalt was administered as chloride. When labeled cobalt was administered orally to cattle, about 80% of the dosage was eliminated in the feces and about 0.5% in the urine; very little was absorbed and available for general distribution throughout the tissues. Relatively high concentrations in the small intestine and intestinal lymph glands indicated the probable route of absorption. The liver was the chief storage organ; the liver of an animal sacrificed 5 days after cobalt ingestion containing 0.4% of the dose.

[156] D. M. Greenberg, D. H. Copp, and E. M. Cuthbertson, *J. Biol. Chem.*, **147** 749 (1943).
[156a] C. L. Comar and G. K. Davis, *Arch. Biochem.*, **12, 257** (1947).

When labeled cobalt was injected into the jugular vein of cattle, the disappearance from the blood was rapid; about 65% of the dose was eliminated in the urine and about 30% in the feces. There was general distribution throughout the tissues; considerable and rapid deposition of cobalt, up to 46% of the dose, occurred in the liver with subsequent excretion via the bile. Higher concentrations were found in the glandular organs, particularly the adrenals, thyroid, liver, thymus, intestinal lymph glands and pancreas. Small amounts of injected cobalt were found in the abomasum contents and practically none in the contents of the other stomach compartments.[156a] The tissue distribution of radioactive cobalt injected into the jugular vein of a month-old calf was similar to that for mature animals. A comparison of similar data on swine, rabbits, and cattle showed no results which would indicate a species difference in regard to the internal metabolism of cobalt.[156b]

TABLE 57

Distribution of Manganese in Organs of the Rat[157]

Organ	Per cent of administered ^{56}Mn present in 1 g. fresh tissue			
	Time, hr.			
	0.5	1	2	6
Blood	0.21	0.13	0.05	0.11
Liver	1.83	1.98	3.45	1.34
Kidney	0.78	1.08	0.99	0.38
Spleen	0.15	0.15	0.10	0.04
Thymus	0.21	0.27	0.28	0.48
Testes	0.17	0.13	0.15	0.03
Brain	0.01	0.03	0.03	0.06
Adrenals	0.21	0.31	0.25	0.31
Thyroid	0.65	0.71	0.77	0

Extremely small but definite amounts of radioactive cobalt injected or fed to pregnant cows were transmitted across the placenta for storage in the liver of the fetus.[156a]

0.3% of the radiocobalt injected into a dog appeared in the external secretion of the pancreas.[156c]

The distribution of manganese, determined by Born and co-work-

[156b] C. L. Comar and G. K. Davis, *J. Biol. Chem.*, **170**, 379 (1947).

[156c] G. E. Sheline, I. L. Chaikoff, and M. L. Montgomery, *Am. J. Physiol.*, **145**, 285 (1946).

ers,[157] in the organs of the rat at different times after intravenous injection is shown in Table 57. Liver, kidney, and thyroid were found to be most effective in retaining radiomanganese (^{56}Mn).

Radiomanganese was used to study perosis produced in chicks by a synthetic manganese-deficient diet. The major part of the labeled manganese, whether administered orally or parenterally, was found to be excreted by the deficient as well as by the control chicks.[158]

XXII. Copper

The maximum radiocopper content in the plasma of a dog, after consumption of radiocopper with a meal, was found by Joshikawa and associates[159] at 2 to 4 hours after administration; after 48 hours, radiocopper could still be detected. The corpuscles showed an increase up to 2 days. In anemic dogs, the uptake of radiocopper in the corpuscles was found to be accelerated.

After feeding 0.1 mg. labeled copper to rats, most of the copper was recovered from the gastrointestinal tract and the excreta. With respect to urinary excretion, copper behaves much like manganese, and unlike cobalt (see page 179). The distribution of copper fed to anemic rats is shown in Table 58. The kidney, the liver, and the bone marrow show

TABLE 58

Distribution of Radiocopper Fed to Anemic Rats[160]

| Organ | Deficiency of | |
	Copper	Iron
Blood	0.54[a]	0.16[a]
Liver	1.57	0.32
Kidneys	0.56	0.32
Spleen	0.14	0.02
Bone marrow	0.05	0.01
Carcass	2.54	1.43
Gastrointestinal tract + excreta	70.00	74.93
Retained	5.40	2.34

[a] Results expressed in per cent of radiocopper fed (100 to 150 mg. per rat). Duration of experiment, 24 hours.

[157] H. J. Born, H. A. Timoféeff-Ressowsky, and P. M. Wolf, *Naturwissenschaften*, **31**, 246 (1943).

[158] M. S. Mohamed and D. M. Greenberg, *Proc. Soc. Exptl. Biol. Med.*, **54**, 197 (1943).

[159] H. Yoshikawa, P. F. Hahn, and W. F. Bale, *J. Exptl. Med.*, **75**, 489 (1942).

the highest retention of copper at 24 and 48 hours. The entrance of copper into the hematopoietic centers in amounts too small to permit detection by chemical methods has been demonstrated in these experiments. This is an interesting finding in view of the fact that, when copper is fed to an anemic, copper-deficient rat, the effects on the hematopoietic organs are quickly noticeable, most striking being the immediate increase in cytochrome oxidase activity of the bone marrow from very low to normal or even above normal. Copper-deficient rats retain more of a single therapeutic dose of copper than do iron-deficient rats.[160]

Schubert *et al.*[160a] found that 10.5 hours after intravenous injection of radiocopper to the dog 1.6 and 1.2 microcuries were present in the red corpuscles and plasma, respectively. The lowest radiocopper content was shown by brain and thymus, and the highest by the liver. The cortex of the kidneys showed high values as well. Nearly all the radiocopper present in the plasma is bound to protein,[160b] but only a small amount is tied to the globulin fraction.

TABLE 59

Relative Distribution of Gold in Organs of a Rat after Intravenous Administration[a] of Radiogold[160c]

Organ	Time after injection		Organ	Time after injection	
	2 days	8 days		2 days	8 days
	Counts/min./g. wet tissue			Counts/min./g. wet tissue	
Kidney....	100.0	100.0	Small intestine.....	6.22	13.20
Spleen.....	50.0	53.50	Articular cortex		
Adrenals...	17.6	47.60	(bone)..........	19.90	12.00
Marrow....	12.52	29.80	Heart............	5.11	11.00
Liver......	12.80	27.60	Aorta............	10.61	6.08
Synovia....	6.50	24.50	Spinal cord........	0.92	2.95
Lung......	11.42	19.00	Muscle...........	2.56	2.81
Tendon....	6.28	13.70	Compact bone.....	5.77	1.26
Blood ...	11.00	13.50	Aqueous humor....	2.55	1.11
			Brain............	0.7	0.93

[a] As gold sodium thiosulfate containing 0.5 mg. gold, activity 142 μc.

[160] M. O. Schultze and S. J. Simmons, *J. Biol. Chem.*, **142**, 97 (1942).
[160a] G. Schubert, H. Vogt, W. Maurer, and W. Riezler, *Naturwissenschaften*, **31**, 589 (1943).
[160b] H. Yoshikawa, P. F. Hahn, and W. F. Bale, *Proc. Soc. Exptl. Biol. Med.*, **49**, 285 (1942).

XXIII. Gold

The distribution of gold in various perfused organs of the rabbit was investigated by Tobias *et al.*[160c] in the course of a study of the application of organic gold salts in the treatment of rheumatoid arth-

Fig. 38. Radioautograph of a rat kidney. The rat was sacrificed 48 hours after receiving 560 μc. [198]Au in the form of gold sodium thiosulfate (1.0 mg. Au).[160c]
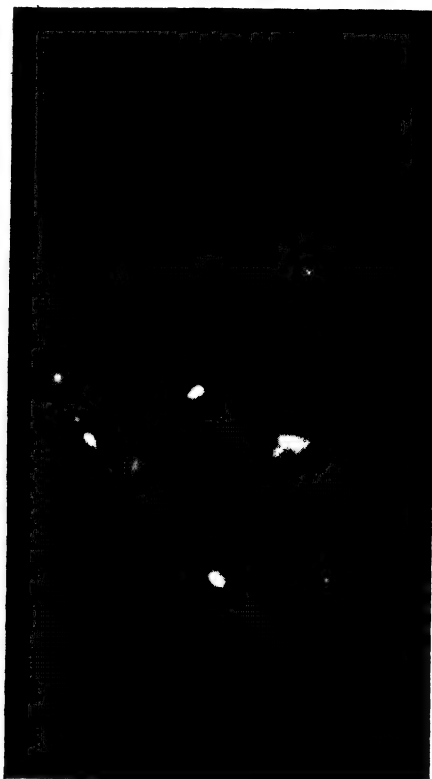
Fig. 39. Section through lateral ventricles of the brain of a white rat showing gold in the cortical tissue and accumulated in the blood vessels.[160] The animal received 560 μc. [198]Au as gold sodium thiosulfate 24 hours prior to killing. *Courtesy* Dr. J. B. Tobias.

ritis. The results of an experiment in which gold thiosulfate containing 0.5 mg. gold with an activity of 0.142 millicurie was injected into the

[160c] C. A. Tobias, J. J. Bertrand, and J. Waine, *personal communication.*

veins of the rabbit is seen in Table 59. Beside the kidneys, spleen, adrenals, marrow, and liver the synovial membrane is found to take up an appreciable amount of the gold administered. This is a result of interest, since inflammation of this membrane is one of the outstanding symptoms of rheumatoid arthritis. The distribution of toxic amounts of radiogold in rats 6 to 66 hours after intraperitoneal and subcutaneous administration was investigated previously.[160c] Some results of these investigations are seen in Figures 38 and 39, showing radioautographs of the kidney and brain, respectively. Radiogold as the chloride was found to be absorbed relatively slowly and to accumulate in all tissues.[160d]

XXIV. Zinc

Data on the distribution and excretion of zinc were obtained by Sheline *et al.*[161,162] by injecting 1 to 12 μg. zinc chloride intravenously into mice and dogs. Radiozinc prepared from the copper deflector of the Berkeley cyclotron was used in the study of the circulation of zinc of negligible weight. From zinc intravenously injected into the mouse, as much as 50% was eliminated by way of the gastrointestinal tract within 170 hours. In the dog, about 25% was found in the feces at the end of 12 to 15 days. Labeled zinc appeared early in the urine of both mice and dogs, and continued to be excreted throughout the period of observation, 170 hours in the case of the mice, and 15 days in the case of the dogs. The amounts of ^{65}Zn eliminated by this route were small compared with the quantities excreted in the feces.

Per gram of tissue, the pancreas was found most effective in taking up zinc, followed by liver and kidney (see Figures 40 and 41). The amount taken up by these organs declines appreciably with time. At the 3-hour interval, 38% of the radiozinc is found in the dog's liver, while only 3.5% is present in this organ at the 170-hour interval. These observations suggest that the ^{65}Zn lost by the liver between 3 and 170 hours is deposited in other tissues. Similar behavior is shown by many other substances. Even potassium introduced in the circulation shows similar behavior (see page 223).

[160d] J. O. Ely, *J. Franklin Inst.*, **230**, 125 (1940).

[161] G. E. Sheline, I. L. Chaikoff, H. B. Jones, and M. L. Montgomery, *J. Biol. Chem.*, **147**, 409 (1943); **149**, 139 (1943).

[162] M. L. Montgomery, G. E. Sheline, I. L. Chaikoff, *J. Exptl. Med.*, **78**, 151 (1943).
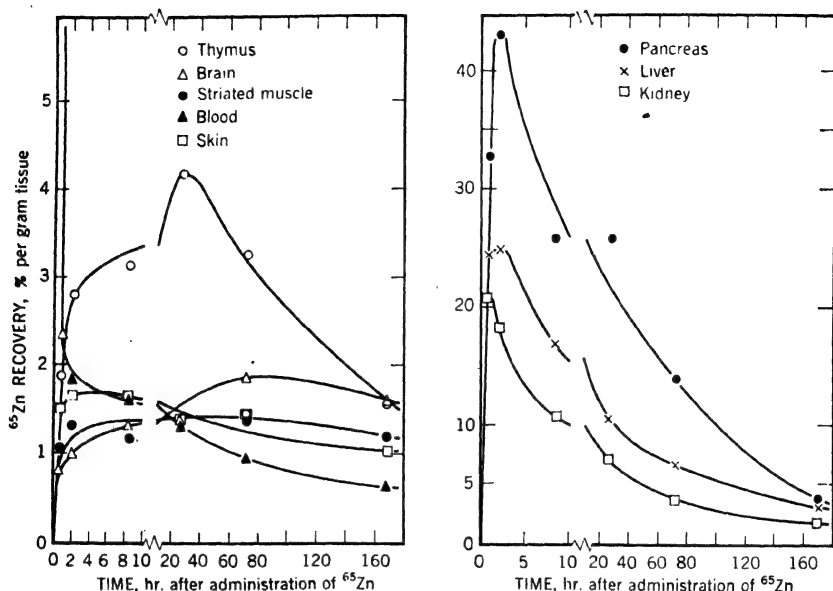
Fig. 40
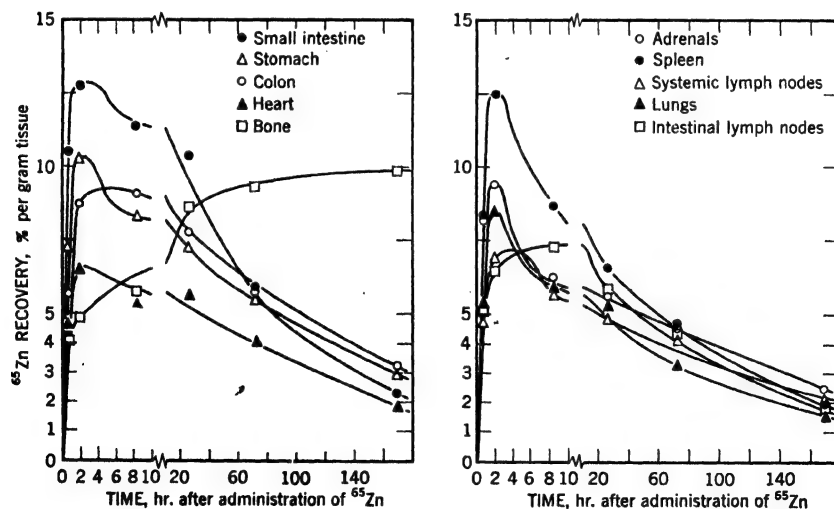
Fig. 41

Figs. 40 and 41. Recovery of intravenously injected [65]Zn in organs of the mouse.[161] Each value is the average of three to seven separate determinations on as many animals. The mice weighed 18–23 g. and each received 0.31–1.6 μg. of labeled zinc.

Evidence has been presented[162] for the view that the acinar portion of the pancreas is concerned with the metabolism of zinc. It was shown with the aid of labeled zinc (^{65}Zn) that as much as 11% of administered zinc was eliminated by the pancreatic juice of dogs within 14 days. While the maximum excretion observed in the bile was only 0.4% in 8 days, large amounts were observed in the juice obtained from an isolation loop of the duodenum. Subcutaneous and intramuscular injections of radiozinc suspended in pectin solution are not followed by diffusion of radioactivity outside the injected areas, as shown both by radioautographs and by counting the radioactivity of blood specimens. The use of such suspensions for the production of localized biological radiation has been proposed.[163]

XXV. Inert Gases

The early rate of gas uptake by the organism is generally considered to be determined largely by the cardiovascular condition of the animal. This point was tested by making use of radiokrypton as a tracer.[164] A tube was inserted into the trachea of a dog and made air-tight by inflating a rubber cuff. The tube was then connected to the spirometer containing the labeled gas–oxygen mixture. The rate of gas uptake was subsequently measured by a Geiger counter placed in contact with the right hind foot, both enclosed in a lead-walled chamber. Concentration of the gas–oxygen mixture was maintained at a constant level.

As a criterion of gas uptake by the animal an index was developed. It consists of drawing a curve through the points obtained by plotting counts per minute against time, and taking the ratio of the number of counts at 7 minutes to the number of counts at 14 minutes. Good reproducibility is observed, the average figures for 5 dogs varying between 61.4 and 70.0.

An index lower by 11% is observed when the animal is infused with large quantities of normal saline; warming the whole body increased the index 5%, and warming the foot, 12%. When the body was chilled to shivering, a decrease of the index by 9% was found to take place.

Adrenaline, histamine, and dexedrine were found to lower the index, while xanthines, which tend to facilitate peripheral blood flow, raise it. Uptake of krypton and its disappearance from the hand and knee region

[163] J. H. Müller, *Experientia*, **2**, 372 (1946).
[164] S. F. Cook and W. N. Sears, *Am. J. Physiol.*, **144**, 164 (1945).

was studied by Tobias *et al.*[164a] using radiokrypton (half-life = 34 hours). Radionitrogen (half-life = 9.9 minutes) was also applied in these experiments. After 10 minutes of strenuous exercise the rate of krypton uptake by the hand was appreciably larger than in the resting controls. Following exercise, 5 minutes after the radiokrypton was inhaled, 33% of saturation was obtained, in the controls only 14%.

Appreciable amounts of radon applied in ointments are absorbed through the intact skin, and still more through open wounds.[164b]

XXVI. Air-Borne Fission Products

Interesting results were obtained relating to the metabolism, in rats, of air-borne fission products. Four different isotopes were used, selected for their long lives and as representatives of the various chemical species occurring in fission. These were ^{89}Sr (55 days), ^{95}Zr (65 days), ^{91}Y (57 days), and ^{144}Ce (275 days). Aerosols of these materials were produced by atomizing aqueous solutions and by burning dried residues in a carbon arc.

Strontium differs from the other elements investigated in the ease with which it is absorbed from the lungs. Over 50% left the lungs within a matter of minutes, and 95% was gone within 1 hour. Of the ^{89}Sr originally in the lung, 60% was deposited in the skeleton in less than 4 hours, and after 12 hours the total radiation dose to the skeleton exceeded that to the lungs. These results indicated that inhalation was as effective a means of administration as intravenous injection, and that lung damage as a result of inhalation of ^{89}Sr would be of less importance than bone and bone marrow damage.

The other three elements, ^{91}Y, ^{95}Zr, and ^{144}Ce, may be grouped together insofar as they differ from ^{89}Sr. They were all eliminated from the lung much more slowly than was Sr. Thus the times required for 90% elimination were: ^{91}Y, 12 days; ^{95}Zr, 25 days; and ^{144}Ce, 45 days. In all cases the skeleton was the major site of deposition and eventually contained between 15 and 30% of the initial lung deposit. In no case, in periods ranging up to 200 days, did the total radiation dose to the bone exceed that to the lung, as it did within 12 hours with ^{89}Sr. With all three elements the probability of radiation damage to the lung is great.

[164a] C. A. Tobias, *personal communication.*
[164b] K. Lange and R. D. Evans, *Radiology,* **48,** 514 (1947).

Very little deposition occurred in the soft tissues. The kidney contained about 0.5% of the dose with all the elements. Concentration in the liver was lower than in the kidney with all isotopes except [144]Ce. This element showed a very high initial liver deposition, but was gradually eliminated so that after 2 months the liver concentration fell below that in the kidney.[164c]

Inhaled ruthenium is rapidly absorbed from the lung (more slowly than iodine, but much more rapidly than zirconium). Within a wide limit of experimental error, there is no evident effect of valence state upon rate of absorption from the lung. Absorbed ruthenium resembles the alkali metals more than any other group of elements in the rapidity with which it is excreted. It shows little tendency to deposit in any organ and is quickly eliminated by both urinary and fecal routes. The concentration of ruthenium in the kidney is usually somewhat higher than in other tissues, and there is some indication that kidney deposition is more pronounced with RuO_4 than with the lower valence states, although this is not certain.[165]

[164c] K. G. Scott and J. G. Hamilton, *in press.*
[165] M. Dailey, J. Wender, and R. Abrams, *in press.*

Application of Isotopic Indicators in the Study of Permeability of Phase Boundaries

I. General Remarks

The organism is an aggregate of almost innumerable phases. The boundaries of these phases are endowed with a remarkably selective permeability to metabolites. This property of the phase boundaries permits the cells to prevent leakage of indispensable substances, and makes possible the entrance of compounds essential for the cell function and the elimination of waste products.

Isotopic indicators prove to be very useful in the study of permeability problems. We shall first consider some investigations of the permeability of the capillary wall.

II. Rate of Ion Passage through Capillary Wall

The substances absorbed into the circulation will sooner or later leave the plasma through the capillary wall, an alternative place of refuge being offered by the corpuscles. We shall first discuss the rate of passage of ions through the capillary wall.

The partition of a substance introduced into the circulation between the plasma and the extracellular fluid involves two processes: penetration across the capillary wall, and distribution by diffusion and convection processes in the capillary and the extracellular fluids. The last-mentioned processes will play a secondary part only, in view of the very short distances between the capillaries. Taking the length of the distances involved to be less than 60 μ and the diffusion coefficient of the substance investigated to be about one square centimeter per day, the time necessary to displace, for example, a sodium ion from one end of the capillary space to the other or from one end of the corresponding extracellular space to the other will be less than two seconds. We arrive at this result by considering the propagation by diffusion alone

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of the substance which has penetrated the capillary wall. The fluid is, however, not without a circulation of its own, and this will possibly shorten the time found in the above calculation.

By introducing some sodium chloride into the circulation and measuring the time taken by a given fraction to leave the circulation, it should be possible to measure the rate of passage of sodium chloride through the endothelium. However, when carrying out these experiments we meet with the following difficulties: (a) The circulation eliminates excess sodium chloride not only by giving off salt to the extracellular space, but also by taking up water from the tissues. It was found,[1] for example, when studying the fate of sucrose intravenously injected into man, that osmotic equilibrium is attained by a shift of water from three to ten times as fast as by sucrose exchange. The rate of disappearance of the excess sodium chloride will thus reflect not the rate of passage of sodium chloride through the capillary wall, but of a more complex process. (b) When using the method outlined above we do not measure the rate of passage of sodium through the endothelium but a resultant of the rates of passage of sodium and chloride. The resistances of the endothelium to the passage of these two ions may be quite different. (c) The introduction of appreciable amounts of sodium chloride into the circulation will disturb the normal conditions prevailing there. When one attempts to eliminate this difficulty by introducing small amounts only, the analytical difficulties become almost insurmountable. All these difficulties can be circumvented by injecting into the veins labeled sodium chloride (sodium chloride containing radioactive ^{24}Na) of negligible weight and by measuring the rate of disappearance of the active ions from the plasma, i.e., the decrease in the radioactivity of the plasma. In these experiments we are not determining the rate of influx of excess sodium chloride from the plasma into the extracellular fluid but the rate of exchange between labeled plasma sodium and nonlabeled extracellular sodium. The rate of exchange will be determined by the permeability of the capillary wall to sodium ions and will thus constitute a measure of this permeability.

Similar experiments were carried out with the radioactive isotopes of potassium, rubidium, strontium, chlorine, bromine, and other elements. Some of the results obtained are seen in Tables 60 to 63 and Figures 42 and 43. In the figures, the ordinates represent the volume of diluting fluid necessary to bring down the concentration of the sub-

[1] A. Keys, *Trans. Faraday Soc.*, **33**, 972 (1937).

stance injected to that found after a given time. The diluting volume is expressed in per cent of the body weight of the rabbit.
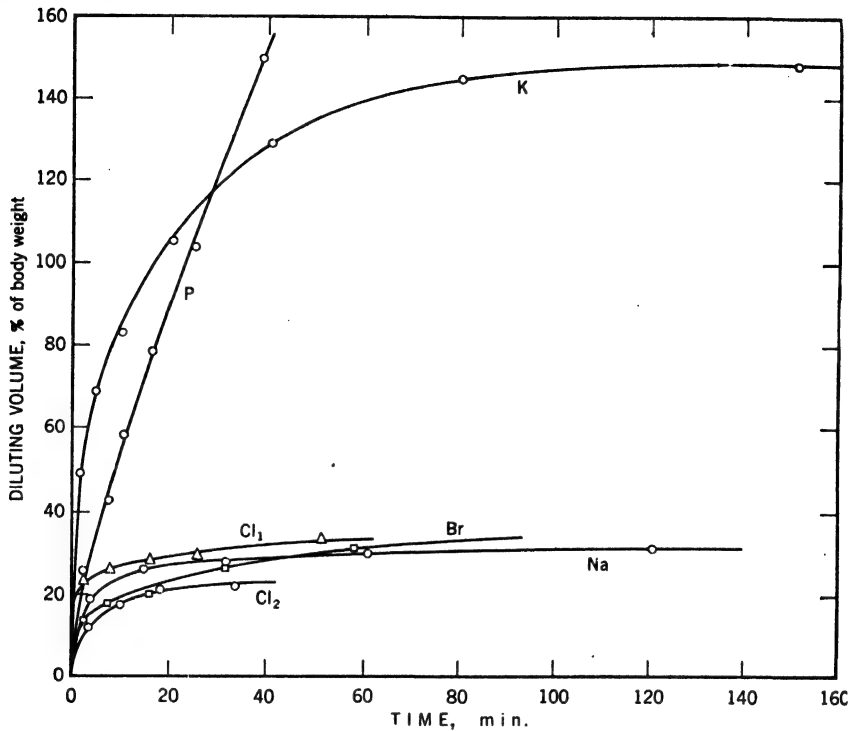
Fig. 42. Rate of disappearance of various labeled ions from plasma.[2]

TABLE 60

Rate of Disappearance of ^{24}Na from the Circulation of a 2.7-Kilogram Rabbit[2]

Time, min.	Per cent of injected ^{24}Na in 1 g. plasma	Diluting fluid volume	
		Apparent extracellular volume, ml.	Per cent of body weight
0.2	0.80	125	4.6
0.45	0.50	199	7.4
0.9	0.41	242	9.0
1.5	0.32	310	11.5
2.2	0.30	331	12.3
3.8	0.234	427	15.8
5.2	0.215	466	17.8
11	0.194	515	19.1

[2] L. Hahn and G. Hevesy, *Acta Physiol. Scand.*, **1**, 347 (1941).

We shall first compare the rates of disappearance of sodium, chlorine, and bromine from the circulation. This comparison encounters no difficulties since almost the sole outlet for these elements from the circulation is the extracellular body fluid; some ^{24}Na, however, is taken up by the surface layer of the bone apatite (Chapter X), by the red corpuscles, and to a minor extent by the cells of some organs (see page 227). In experiments lasting up to 1 hour, the amount of ^{24}Na lost by excretion is less than 1% of the amount administered. No great difference is found between the rates of passage of sodium,

Fig. 43. Change with time of ^{24}Na concentration in guinea pig plasma.[3]

chlorine, and bromine through the capillary wall. When interpreting the figures obtained for the rate of disappearance of labeled ions from the plasma in experiments of a few minutes or less, it must be remembered that the ions were injected into the jugular vein, and the blood samples were obtained from the carotid artery. As discussed on page 215, mixing of the ions in the plasma takes about one minute.

A quantitative comparison of the rates of passage of potassium, phosphate, and water with those of sodium, chlorine, and bromine meets with difficulties since potassium, and also phosphate and water, in contrast to the latter group, have an important additional outlet into the tissue cells. The amount of ^{42}K found after the lapse of a given time in the circulation is the result not only of the amount which has penetrated the tissue fluids and returned from the latter into the blood, but is also influenced by the amount which has entered the cells. When, in addition to the interspaces, the intracellular space opens an outlet to the ^{42}K leaving the circulation, the amount returning from the tissue fluids into the blood will be reduced and the resultant ^{42}K concentration of the plasma will thus be lowered. In view of the low potassium content of the extracellular fluid and the high potassium content of the tissue cells, the additional outlet provided by the entrance of ^{42}K into the cells takes from the circulation several (about fifty) times more ions than penetrate into the interspaces; similar considerations apply to water and phosphate. Since the volume of the total body water amounts

[3] M. Merrell, A. Gellhorn, and L. B. Flexner, *J. Biol. Chem.*, **153**, 83 (1944).

to about three times the volume of extracellular water, the rapid penetration of labeled water into the cells provides a substantial additional outlet for the labeled water molecules of the plasma.

Merrell and associates[3] calculated from their results seen in Figure 43 that 60% of the plasma sodium and 13% of the sodium in the extravascular fluid were transferred per minute in either direction across the vascular membrane of the guinea pig. In carrying out the calculation they assume that the amount of ^{24}Na lost from the plasma per unit time is proportional to the number of milligrams of sodium that move from plasma to extravascular fluid per unit time and to the proportion of the sodium in the plasma which is radioactive. Part of the ^{24}Na that escapes into the extravascular fluid will return to the plasma and the amount that returns per unit time is proportional to the amount of sodium that moves from the extravascular fluid to the plasma per unit time and to the proportion of the extravascular sodium that is radioactive. These considerations lead to the equation:

$$C_p - C_{eq} = (C_0 - C_{eq})e^{-(R/q)t}$$

where C_p = concentration of ^{24}Na in the plasma, C_{eq} = the concentration of ^{24}Na in the plasma at equilibrium, R = proportion of the plasma sodium which escapes from the plasma into the extravascular fluid per unit time, and q = the proportion of total sodium that is extravascular.

Gellhorn and co-workers[4] determined, furthermore, whether traumatic shock produced an alteration in the normal exchange of sodium ions across the vascular membrane. Their investigations showed that in untreated traumatic shock the total number of milligrams of sodium exchanged across the vascular membrane per unit time is about 50% of normal. Following replacement therapy with saline or serum in traumatic shock, the total amount of sodium passing back and forth between the plasma and the extravascular fluids is still only about 50% of the normal value.

A comparison of the rates of migration of sodium and labeled water across the membrane has been made as well. Twice as much water was found to cross the vascular wall per minute as was to be expected from their relative concentrations in the plasma.

Greenberg et al.[5] found that the rate of disappearance of the injected

[4] A. Gellhorn, M. Merrell, and R. M. Rankin, *Am. J. Physiol.*, **142**, 407 (1944).

[5] D. M. Greenberg, R. B. Aird, M. D. D. Boelter, W. Wesley, W. W. Campbell, W. E. Cohn, and M. M. Murayama, *Am. J. Physiol.*, **140**, 47 (1943/44).

labeled ions from the blood plasma occurred in the order: K = Rb > P > I > Na > Br > Sr. However, since the ions of an element injected and those previously present in the plasma can be assumed to show the same behavior and the sodium concentration in the plasma is about 20 × higher than the potassium concentration, the amount of sodium leaving the circulation per unit time is appreciably higher than that of potassium.[6]

After the lapse of 3 minutes, 40% of the $^{65}ZnCl_2$ injected into the veins was found to be present in the plasma of the dog. After the lapse of 30 minutes, 3% remained, while, after 10 hours, the injected radiozinc had practically disappeared from the plasma.[7]

The divalent ions of the radium isotope thorium X were found to pass with great rapidity through the capillary wall; after 40 seconds the blood of human subjects was found to contain only 30% of the thorium X injected.[7a]

TABLE 61

Rate of Disappearance of ^{42}K from the Circulation of Rabbits[2]

Time, min.	Per cent of injected ^{42}K in 1 g. plasma	Diluting fluid volume	
		Apparent extracellular volume, ml.	Per cent of body weight
Rabbit 1 (2.5 kg.)			
0.6	0.19	526	21
2.0	0.082	1220	49
3	0.068	1470	59
5	0.058	1720	69
15	0.035	2860	114
Rabbit 2 (2.4 kg.)			
5	0.058	1730	72
10.5	0.048	2080	87
20.5	0.038	2640	110
40.5	0.031	3220	134
80	0.0277	3610	151
210	0.0269	3720	155

[6] G. Hevesy, L. Hahn, and O. Rebbe, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **16**, No. 8 (1941).

[7] G. E. Sheline, I. L. Chaikoff, H. B. Jones, and M. L. Montgomery, *J. Biol. Chem.*, **149**, 139 (1943).

[7a] J. Gerlach, *Naturwissenschaften*, **29**, 300 (1941).

TABLE 62

Rate of Disappearance of ^{38}Cl from the Circulation of a 2.5-Kilogram Rabbit[2]

Time, min.	Per cent of injected ^{38}Cl in 1 g. plasma	Diluting fluid volume	
		Apparent extracellular volume, ml.	Per cent of body weight
0.37	0.622	161	6.4
0.73	0.486	206	8.2
1.01	0.475	211	8.5
1.48	0.408	245	9.8
2.05	0.400	250	10.0
3.8	0.329	304	12.2
10.5	0.224	446	17.8
18.5	0.188	532	21.3
35	0.182	550	22.0

TABLE 63

Rate of Disappearance of ^{32}P from the Circulation of Rabbits[2]

Time, min.	Per cent of injected ^{32}P in 1 g. plasma	Diluting fluid volume	
		Apparent extracellular volume, ml.	Per cent of body weight
Rabbit 1 (2.1 kg.)			
1.1	0.300	333	15.9
1.9	0.234	427	20.4
3.0	0.187	535	25.5
4.5	0.143	699	33.3
6.8	0.112	892	42.5
10.9	0.081	1230	58.6
16.9	0.060	1670	79.5
25.9	0.046	2180	104
39.0	0.032	3130	149
Rabbit 2 (2.7 kg.)			
0.2	0.63	160	5.9
0.45	0.46	216	8.0
0.9	0.35	287	10.6
1.5	0.261	383	14.2
2.2	0.234	428	15.9
5.2	0.120	835	30.9

III. Permeability Studies with Heavy Water

One might carry out an experiment with heavy water as follows: Inject a few milliliters of practically pure heavy water into the jugular

vein of a rabbit and take blood samples at intervals from the carotid. Then prepare pure water from the blood samples and determine its density. Let us assume that we inject 1 ml. heavy water with a density of 1.1000 and find for the water prepared from a blood sample the value 1.001. We must conclude that the 1 ml. heavy water injected into the vein was diluted with 99 ml. normal water present in the body of the rabbit during the period elapsing between the injection of the heavy water and the collection of the blood sample.

In an experiment[8] the results of which are seen in Table 64, 5.0 ml. heavy water was injected into a rabbit weighing 1.5 kg. The injection lasted 6 seconds. The first blood sample was taken in the interval 22–26 seconds after the start of the experiment. The time recorded is reckoned from the moment when half the heavy water had been injected until the time at which half the blood sample had been collected.

TABLE 64

Rate of Disappearance of Heavy Water from the Circulation of the Rabbit[8]

Time	Density excess of blood water, p.p.m.	Extent of dilution of 1 ml. heavy water in circulation	Diluting water volume, per cent of body weight
21 sec.	1034	506	34
80	856	612	40.8
1.8 min.	794	661	44.1
3	719	727	47.8
5.2	570	921	61.4
7.9	495	1056	70.4
13	490	1070	71.3
22.2	440	1190	79.4
30	450	1167	77.9
48 hr.	412	1274	85

In this and other experiments,[2] it was found that within about 0.5 minute labeled water injected into the jugular vein of a rabbit was diluted with a large amount of body water, the volume of which corresponds approximately to the extracellular space of the body. This rapid dilution was followed by a second, slower process, presumably due mainly to a further dilution of the heavy water with cellular body water. From these findings it follows that the water molecules present in the plasma pass with remarkable speed through the capillary and also the cell wall,

[8] G. Hevesy and C. F. Jacobsen, *Acta Physiol. Scand.*, **1**, 11 (1940).

although it takes about 20 minutes for injected water to reach exchange equilibrium with the total water content of the rabbit. A similar result was obtained in an experiment in which water containing tritium (radioactive hydrogen) was used as an indicator.[8a] However, it may possibly be argued that heavy water (D_2O) may show a different behavior from ordinary water, in view of the results obtained in the investigation of the rate of hemolysis of erythrocytes of cattle and rats — hemolysis was found to take place about 44% more slowly in heavy than in ordinary water. In contradistinction to these investigations,[9] however, the experiments under consideration were carried out not with pure heavy water but with very dilute heavy water, the viscosity and other properties of which differ only slightly from those of ordinary water. The most concentrated samples contained, in fact, less than 2% heavy water, most of them having very much smaller concentrations. The heavy water injected into the vein is diluted at once. When heavy water is used as an indicator it should always be used in as great a dilution as possible, for the above reasons, and because such diluted solutions contain mainly DHO, which is very similar to H_2O, while D_2O is much less so.

TABLE 65

Distribution of Heavy Water between Blood and Organs of the Mouse and Rat[10]

D₂O–Ringer injected, g.	Time after injection, min.	Density excess of water extracted, p.p.m.			
		Blood	Brain	Muscles	Liver
0.25 into tail vein of mouse..	0.5	1993	1900	1567	——
0.25 into tail vein of mouse..	1	2013	2044	1397	——
0.2 into tail vein of mouse..	4	1723	1775	1721	——
0.5 into jugular vein of ether-narcotized rat..........	0.5	458	170	19	——
0.4 into jugular vein of ether-narcotized rat..........	1	376	320	150	354

The rate of distribution of heavy water between blood water and water extracted from brain and muscles of the mouse and the rat was investigated by Krogh and Ussing.[10] As is seen in Table 65, heavy

[8a] N. Pace, L. Kline, H. K. Schachman, and M. Harfenist, *personal communication.*

[9] A. K. Parpart, *J. Cell. Comp. Physiol.,* **7,** 153 (1935). S. C. Brooks, *ibid.,* **7,** 163 (1935).

[10] A. Krogh and H. H. Ussing, *personal communication.*

water is almost instantly distributed between the water isolated from the blood and the brain. McDougall *et al.* determined the distribution of heavy water in rats.[10a]

Flexner and colleagues[11] calculated from the rate of disappearance of heavy water from the blood of the guinea pig that a volume of water equal to 73% of the water content of the blood flows from the circulation into the extravascular fluid per minute and vice versa. They also found that the mixing of the labeled water injected into the circulation was at least 95% complete at the end of 1 minute and 100% complete after 3 minutes.

IV. Determination of Total Body Water by Use of Radioactive Hydrogen

The determination of the total body water content of human subjects was carried out by Pace *et al.*[8a] by making use of water containing radioactive hydrogen (tritium). The 5.09 ml. water injected in the human experiment had an activity of 9×10^9 counts per minute (the preparation of tritium is described on page 14 and its determination on page 55). The labeled water was introduced into the antecubital vein of a 19 year old normal male subject, 184 cm. tall and weighing 70.8 kg. At intervals of 30 minutes and 1, 2, and 3 hours, blood samples

TABLE 66

Determination of Total Body Water of Four Humans[8a]

Time after labeled water injection, hr.	Activity of 1 ml. plasma water, per cent of activity of 1 ml. injected water	Body water, per cent
0.5	0.0099	71.9
1.0	0.0114	62.2
2	0.0106	67.6
3	0.0111	64.3

were withdrawn from the antecubital vein and the plasma was stored for tritium activity measurement. Total body water content was calculated making use of the formula $A_1 \times V_1 = A_2 \times V_2$. A_1 is the activity of tritium in the stock solution, V_1 is the volume in milliliters of the stock solution injected, A_2 is the activity of tritium in a body

[10a] E. J. McDougall, F. Verzar, H. Erlenmeyer, and H. Gaertner, *Nature*, **134**, 1008 (1934).

[11] L. B. Flexner, A. Gellhorn, and M. Merrell, *J. Biol. Chem.*, **144**, 35 (1942).

fluid, *e.g.*, plasma or urine, after equilibrium has been established, and V_2 is the volume in milliliters of body water. V_2 may be converted to units of weight by multiplying by the density of water at 38°, 0.993, and may then be expressed as a fraction of the total body weight. The results obtained are seen in Table 66. By using heavy water in earlier investigations, 63%[11a] and 72.5%[11b] were recorded for the total body water content. As seen from Table 66, after the lapse of 1 hour the bulk of the labeled water injected reached exchange equilibrium with the body water.

V. Determination of the Extracellular Phase

Manery and Hastings[12] presented evidence that, for the purpose of an approximate description of tissues, at least three chemically different phases are necessary. These phases, which also have morphological significance, are: (*1*) an extracellular phase, which is in ionic equilibrium with the blood plasma and consists essentially of plasma ultrafiltrate and connective tissue proteins; (*2*) an intracellular phase that contains no Na or chloride in appreciable amounts (*e.g.*, muscle fibers); (*3*) an intracellular phase, that contains chloride and may or may not have Na in equivalent proportions (*e.g.*, blood corpuscles, connective tissue cells). Although the relative proportions of these three phases in different tissues cannot, at present, be expressed in quantitative terms except in a few instances, it is of interest to know the extent of the apparent extracellular phase in the organs and in the total organism.

The fact, discussed on page 193, that radiosodium and radiochloride injected into the veins attain exchange equilibrium within a short time with the sodium and chloride present in the organism is precisely what we should expect of elements mainly confined to the extracellular space. By determining the percentage uptake by the various organs of radiochloride or radiosodium administered, we can conveniently obtain figures for the percentage extracellular volume of the organ. The same consideration applies to the total extracellular volume of the body.

A. THE CHLORIDE SPACE

Although the half-life of radiochlorine (^{38}Cl) is only 37 minutes, we can follow the fate of radiochloride for several hours, a time amply

[11a] G. Hevesy and E. Hofer, *Klin. Wochschr.*, **13**, 1524 (1934).
[11b] F. D. Moore, *Science*, **104**, 157 (1946).
[12] J. F. Manery and A. B. Hastings, *J. Biol. Chem.*, **127**, 657 (1939).

sufficient for the determination of the chloride space. Manery and Haege[13] injected a lithium chloride solution with a chlorine activity of about 0.2 millicurie (lithium activity decays with a half-life of 0.9 second) into rabbits via an ear vein, and determined the chloride space of various organs by comparing the activities of a tissue sample and of a plasma sample of known weight. The water space $(H_2O)_E{}^{38}Cl$ is given by the following equation:

$$(H_2O)_E{}^{38}Cl = \frac{\text{activity of 1 g. tissue}}{\text{activity of 1 ml. plasma}} \times 0.95 \times 0.93 \times 100$$

where (H_2O) is expressed in g. per 100 g. fresh tissue, 0.95 is the Gibbs-Donnan ratio, and 0.93 is the correction for the water content of the plasma.

TABLE 67

Chloride Space of the Organs of the Rabbit Determined
by Chemical and by Radioactive Methods[13]

Tissue	Rabbit 1 (2.6 kg.), 11 min.		Rabbit 2 (2.7 kg.), 48 min.	
	$(H_2O)_E$, g./100 g. fresh tissue calculated from			
	Cl	^{38}Cl	Cl	^{38}Cl
Skin	60.6	56	62.6	
Kidney	38.8	41	45.4	59
Liver	19.3	18	21.4	25
Gastrocnemius muscle	11.6	8.3	11.4	9.4
Heart	29.6	31		
Abdominal muscle	15.8	14		
Pyloric muscle	40.4	30	43.8	
Pyloric mucosa		33	58.9	38
Ear cartilage	50.2	37	48.5	58
Tendon	57.1	56		66
Brain	31.8	4.7	33	11

The results obtained are recorded in Table 67, which also contains the values calculated for the extracellular space from the chemical determinations of the chloride content of tissues and plasma. In most tissues the $(H_2O)_E$ values calculated from ^{38}Cl lay close to those calculated from Cl. This fact supports the view that all or almost all

[13] J. F. Manery and L. F. Haege, *Am. J. Physiol.*, **134**, 83 (1941).

chloride present in these tissues is easily interchangeable with extracellular chloride. Relatively small amounts of radioactive chloride are present in the brain tissue 1 hour after injection. This observation falls into line with observations made on the rate of penetration of other ions into the brain tissue, described on page 222. Partition of ^{38}Cl between plasma chloride and organ chloride is obtained in the course of 7–52 minutes except in brain and testes.[14] In pyloric mucosa (and also in testes), the chloride content is greatly in excess of the sodium, making the chloride phase much larger than the sodium phase. The value of $(H_2O)_E$ calculated from ^{38}Cl amounts to only about two-thirds of that calculated from chemically determined chloride and is almost equal to the sodium value. In this tissue (and also in testes), the figures indicate that ^{38}Cl has entered a volume equal to the sodium space and has not progressed beyond it in 52 minutes. Whether the chloride in excess of sodium found by analyses in stomach mucosa occurs within the secreting cells or in the lumen of the glands rather than in the supporting cells is not yet known; at all events plasma chloride does not seem to diffuse freely into this volume or to exchange with the chloride already there.

B. THE BROMIDE SPACE

Investigations of the distribution of bromide between tissue and serum lead to the result that the distribution of bromide is similar to that of chloride in all tissues examined, except the brain and the cerebrospinal fluid.[15] Using radiobromide as a tracer, Perlman et al.,[16] and others[17] corroborated and extended these results. Tracer doses of labeled potassium bromide were administered intraperitoneally to guinea pigs and rats and the distribution of ^{82}Br was investigated 2 to 24 hours later. When the extracellular phase was calculated from the distribution figures, the usual values were obtained for liver, muscle, and adrenal, as seen in Table 68, but not for the thyroid gland. The high values found for the apparent extracellular space of the latter organ clearly indicate that a substantial amount of radiobromide penetrated this gland. The selective activity of the thyroid gland is thus

[14] J. F. Manery, *Am. J. Physiol.*, **129**, 417 (1940).

[15] G. B. Wallace and B. B. Brodis, *J. Pharmacol.*, **65**, 214 (1939). E. G. Weir and A. B. Hastings, *J. Biol. Chem.*, **129**, 547 (1939).

[16] I. Perlman, M. E. Morton, and I. L. Chaikoff, *Am. J. Physiol.* **134**, 107 (1941).

[17] L. Hahn and G. Hevesy, *Acta Physiol. Scand.*, **1**, 347 (1941).

not restricted to iodine and eka-iodine (see page 148). The hyperplastic thyroid, but not the resting colloid-containing gland, contains more bromine than the blood.

TABLE 68

Apparent Extracellular Phase of Organs of the Guinea Pig Based
on Distribution of Labeled Bromine[16]

Time, hr.	Liver	Muscle	Adrenal	Thyroid
2	20.5	20.0	37.5	104
24	23.7	19.1	37.0	122

When the activity of 1 g. gray brain substance was compared with that of 1 g. plasma 59 minutes after administration of radiobromide. the percentage ratio found was 9.3%. Since the chloride space of the brain tissue of the rabbit is 35%, less than one-third the equilibrium partition of bromide between plasma and the extracellular volume of the brain is obtained after 1 hour.[17]

C. THE SODIUM SPACE

The sodium space of the organs is obtained by comparing, 1 hour after the injection of labeled sodium chloride, the activities of an organ sample and a plasma sample of known weight. Manery and Bale[18] in their calculations made use of the formula applied to the determination of the chloride space (see page 202). As seen from the figures of Table 69, injected radioactive sodium becomes distributed between tissues except for nervous tissue) and plasma after the lapse of 1 hour and 8 minutes in precisely the same ratio as ordinary sodium. The entrance into the nervous tissue is much delayed. This is also shown by experiments[17] in which, after the lapse of 2 hours, values of only 14.9% and 10.9% were found for the sodium space of the gray and the white substance, respectively, of the brain. After the lapse of 62 hours following administration of $^{24}NaCl$, however, 31% was found, which corresponds approximately to the sodium space value of the brain tissue. The more rapid uptake of ^{24}Na by the gray substance is probably due to the fact that the vascularity of the gray matter greatly exceeds that of the white.

Making use of the results obtained in their investigation of the distribution of ^{24}Na in rats, Manery and Bale divide the tissues into two

[18] J. F. Manery and W. F. Bale, *Am. J. Physiol.*, **132**, 215 (1941).

groups; those into which the penetration of ^{24}Na is complete in 20 minutes and remains constant for at least 12 hours, and those into which the penetration is delayed but gradually proceeds to completion in 3 to 12 hours. Skin, kidney, liver, and gastrocnemius muscle belong to the former group, and it is probable that abdominal muscle, heart,

TABLE 69

Sodium Space of a Rabbit Injected Intravenously with Radioactive Sodium[18]

Tissue	Per cent dose of ^{24}Na in whole organ	$(H_2O)_B$, g./100 g. calculated from	
		Na	^{24}Na
Skin......................	11.6		
Tendon....................		52.1	53
Kidney....................	1.2	45.8	42
Testes....................		28.4	28
Ear cartilage..............		96.6	104
Spleen....................	0.1	30.1	27
Liver.....................	4.7	22.6	22
Pyloric mucosa.............		33.1	36
Pyloric muscle.............		25.7	29
Small intestine.............	5.1	33.2	32
Sciatic nerve..............	0.2	54.2	28
Cerebrum..................	0.3	35.9	11
Heart.....................	1.1	29.7	30
Abdominal muscle..........	31	17.7	19
Gastrocnemis muscle........		10.0	10
Whole blood...............	17.6		
Rest of body..............	2.1		

and the gastrointestinal tract should likewise be included. The entrance into the gastrocnemius muscle is somewhat delayed. There is some indication that only the chloride space is entered in 20 minutes and that more time is required for penetration of the entire sodium space. When comparing the distribution of thiocyanate and labeled sodium in the determination of the extracellular space in human subjects,[19,19a] higher values were obtained, as seen in Figure 44, with the radiosodium method. Correction of the sodium space of man for the excess sodium

[19] N. L. Kaltreiter, G. R. Meneely, J. R. Allen, S. N. Van Voorhis, and V. F. Downing, *J. Clin. Invest.*, **19**, 769 (1940).

[19a] N. L. Kaltreiter, G. R. Meneely, J. R. Allen, and W. F. Bale, *J. Exptl. Med.*, **74**, 569 (1941).

reduced the average value by 3.7 to 15.9 liters (see Table 71). The average corrected volume for normal subjects 6 hours after the injection was 15.9 liters or 21.1% of the body weight compared with the thiocyanate space of 17.7 liters, representing 23.5% of body weight.
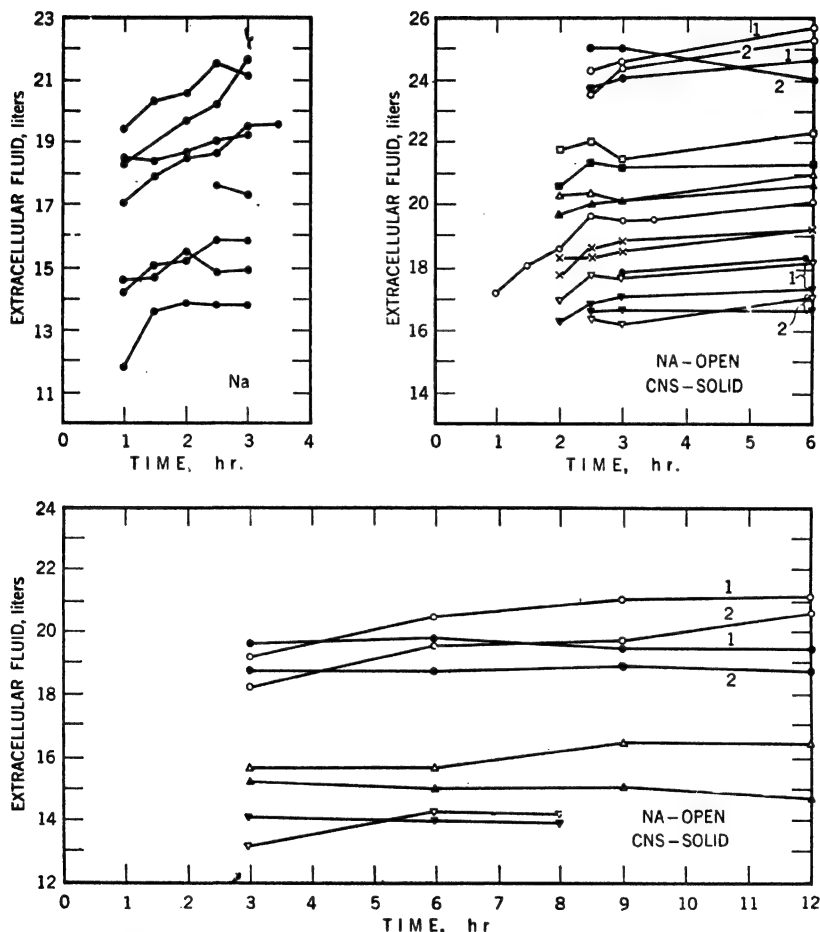
Fig. 44. Rate of diffusion of radiosodium and sodium thiocyanate in the body of normal subjects after intravenous injection.[19]

Values obtained by using radiosodium have to be corrected mainly because the skeleton takes up appreciable amounts of nonextracellular sodium. Sodium atoms presumably replace calcium atoms on the surface layers of bone apatite (see Chapter X). That only about one-third

the radiosodium content of the bones of the dog is due to extracellular sodium is shown by the fact that the chloride space is found to be one-third of the sodium space or less (see Table 70). Similar results were obtained in the study of the uptake of radiosodium by rabbit skeleton.[20] In the table it is also seen that in humans the sodium space of the skeleton is much larger than the chloride space. The sodium space of the human skeleton is estimated to be 50 while that of the chloride space is only 20. Corrected values for the sodium space of human bone tissue are seen in Table 71.

Radiosodium during the first 3 hours after intravenous administration spreads rapidly from the plasma into a volume of fluid which represents approximately 25% of the body weight of man. Thereafter for 6 hours it moves more slowly into certain tissue spaces — the central nervous system and probably the skeleton. Diffusion equilibria for both radiosodium and thiocyanate are not established between serum and transudates in edematous.patients until from 9 to 12 hours after the intravenous injection of these substances (see Table 72).

In the dog following the intravenous injection of radiosodium, the concentration of the isotope in bone reaches its maximum in the course of 3 hours.

In a recent determination of the sodium space, ^{22}Na, which has a half-life of 3 years, was used as an indicator.[21] Rats were raised from the age of 3 to 4 weeks on a chloride-low diet containing 2 to 5 milligram per cent chloride and 2% carbonate and bicarbonate. Such rats had a higher sodium space (33.7) than rats fed a normal diet (26.7), which contained 0.9% chloride and 0.9% carbonate.

In view of the low cellular sodium content of muscle it is very difficult to determine the rate of replacement of the cellular sodium of muscle. When, however, rats are raised on a potassium-deficient diet an appreciable percentage of the muscle potassium is replaced by sodium. In such muscles the ^{24}Na was found to penetrate rapidly the extracellular phase of muscle, as measured by chloride; it took 1 hour to attain exchange equilibrium with the cellular sodium.[22]

An additional outlet for the labeled plasma constituents is provided by their entrance into the corpuscles. For ^{24}Na this outlet is very restricted except in the cases of the dog and the cat. The ^{24}Na content

[20] L. Hahn, G. Hevesy, and O. Rebbe, *Biochem. J.*, **33**, 1549 (1939).
[21] E. M. Cuthbertson and D. M. Greenberg, *J. Biol. Chem.*, **160**, 83 (1945).
[22] L. A. Heppel, *Am. J. Physiol.*, **128**, 449 (1940).

TABLE 70.   Ratios of Bone Concentration to Plasma Concentration

Subject	Compact bone						
	Femur[a]		Scapula	Radius, tibia		Humerus	
	$(H_2O)_E{}^{24}Na$	$(H_2O)_E Cl$	$(H_2O)_E{}^{24}Na$	$(H_2O)_E{}^{24}Na$	$(H_2O)_E Cl$	$(H_2O)_E{}^{24}Na$	$(H_2O)_E Cl$
Dog							
1	66.4	23.5	72.0	—	—	—	—
	59.5	22.1	75.3	—	—	—	—
	66.8	—	—	—	—	—	—
2	56.9	20.1	—	—	—	—	—
	58.4	21.8	—	—	—	—	—
	51.2	—	—	—	—	—	—
	62.2	—	—	—	—	—	—
3	72.6	20.0	—	68[b]	—	62.9	18.2
	—	21.0	—	64.2	—	62.9	—
	—	—	—	68.7[c]	—	—	—
	—	—	—	64.3	—	—	—
	—	—	—	71.4[d]	—	—	—
	—	—	—	75.4	—	—	—
	—	—	—	66.4	20.0	—	—
	—	—	—	64.5	—	—	—
Patient							
1	—	—	—	—	—	—	—
2	—	19.6	—	—	—	—	—
	—	22.8	—	—	—	—	—
			Rib				
3	—	—	51.4	—	—	—	—
	—	—	41.5	—	—	—	—
4	—	—	44.0	—	—	—	—
	—	—	—	—	—	—	—
	—	—	—	—	—	—	—
	—	—	—	—	—	—	—

[a] $(H_2O)_E{}^{24}Na = \dfrac{\text{tissue counts/min./kg.}}{\text{serum counts/min./liter}} \times \dfrac{0.93}{0.95} \times 100$

$(H_2O)_E Cl = \dfrac{\text{tissue Cl (meq./kg.)}}{\text{serum Cl (meq./liter)}} \times 0.93 \times 0.95 \times 100$

for Radioactive Sodium and Chemically Determined Chloride[19a]

Cancellous bone		Compact and cancellous bone		Spinal cord	
Femur, epiphysis, scapula		Vertebra			
$(H_2O)_E{}^{24}Na$	$(H_2O)_E Cl$	$(H_2O)_E{}^{24}Na$	$(H_2O)_E Cl$	$(H_2O)_E{}^{24}Na$	$(H_2O)_E Cl$
45.2	32.9	——	——	——	——
——	26.3	——	——	——	——
53.9	——	——	——	——	——
31.4	17.1	39.9	——	29.3	44.6
36.7	17.7	——	——	——	——
——	——	——	——	——	——
——	——	——	——	——	——
46.3	20.4	51.0	24.4	——	46.0
——	——	——	——	——	——
——	——	——	——	——	——
——	——	——	——	——	——
——	——	——	——	——	——
——	——	——	——	——	——
——	——	——	——	——	——
		Skull			
——	——	48.9	——	——	——
——	——	34.6	——	——	——
——	——	——	——	——	——
——	——	——	——	——	——
——	——	——	——	——	——
——	——	——	——	——	——
——	——	——	——	——	——
		Spine			
——	——	56.5	——	——	——
——	——	62.5	——	——	——

[b] Portion of tibia, diaphysis removed at 3 hours.
[c] Portion of tibia, diaphysis removed at 6 hours.
[d] Portion of radius, diaphysis removed at 9 hours. All other samples obtained 12 hours after injection of radiosodium.

of 1 g. rabbit corpuscles was found (Chapter XI) to be, after the lapse of 2 hours, about 10% that of 1 g. plasma. About one-sixth of the ^{24}Na injected was found in the plasma after the lapse of 2 hours. From

TABLE 71

Fluid Available for Distribution of Radiosodium Corrected for Amount of Extra Sodium in Skeleton (Normal Subjects)[19a]

Subject	Time after injection, hr.	Weight, kg.	Extracellular fluid, sodium space		Extracellular fluid, thiocyanate space,[b] l.	
			Volume, liters	Corrected volume,[a] liters		
5	12	51.0	17.5	15.0	—	—
6	12	69.8	18.4	15.0	—	—
7	6	84.5	20.9	16.9	20.6	19.4
8	6	102.5	22.2	17.3	21.0	19.8
9	6	61.3	18.2	15.2	17.1	16.0
10	6	67.3	18.3	15.1	18.2	17.2
11	6	106.3	25.5	20.4	24.4	22.9
12	6	56.8	15.3	12.6	15.1	14.2
	8	—	15.2	12.5	—	—
13	6	77.6	20.5	16.8	19.7	18.2
	9	—	21.1	17.4	—	—
	12	—	21.2	17.4	—	—
14	6	58.8	15.6	12.8	15.0	14.1
	9	—	16.6	13.8	—	—
	12	—	16.5	13.7	—	—
Average......	6	—	19.6	15.9	18.9	17.7
Body wt., %..	—	—	25.9	21.1	25.0	23.5

[a] Corrected for excess sodium in skeleton.
[b] Determined by two methods.

this figure and the hematocrit value (34%) of rabbit blood it follows that about 0.5% of the ^{24}Na injected finds its way into the corpuscles. The corresponding figure for ^{42}K works out to be about 4%.

The amount of ^{24}Na excreted through the kidneys in the course of 1 hour is negligible (see page 150).

D. DETERMINATION OF THE EXTRACELLULAR FLUID OF THE ORGANISM

From the ratio of the amount of ^{24}NaCl introduced into the plasma and that present after the lapse of a few hours we can calculate the

TABLE 72

Concentration of Radiosodium and Thiocyanate in Serous Effusions as Per Cent of Serum Concentration[19a]

Condition	Time after injection, hr.	Protein, gram per cent		^{24}Na concentration, %	Thiocyanate concentration, %
		Serum	Effusion		
Pleural fluid					
Hodgkin's disease......	3	4.7	2.7	61	101
	6	—	—	87	130
Congestive heart failure.	6	6.4	4.8	90	87
	9	—	—	101	99
	12	—	—	95	101
Congestive heart failure.	6.5	6.4	2.5	103	94
	9	—	—	99	103
Congestive heart failure.	3	6.3	3.0	87	—
	6	—	—	94	—
	24	—	—	97	—
Pulmonary tuberculosis .	9	8.8	6.3	78	78
Synovial fluid					
Rheumatoid arthritis ...	12	7.0	5.4	101	95
Ascitic fluid					
Congestive heart failure.	12	6.3	3.3	95	100
	13	—	—	97	99
Spinal fluid					
Normal...............	3	—	—	22	—
Normal...............	6	—	—	23	9
Normal...............	9	—	—	68	—
Normal...............	12	—	—	101	0

(*Table continued*)

TABLE 72 (concluded)

Condition	Time after injection, hr.	Protein, gram per cent		^{24}Na concentration, %	Thiocyanate concentration, %
		Serum	Effusion		
Saliva					
Normal...............	3	—	—	7	870
Normal...............	3	—	—	7	1150
Normal...............	3	—	—	13	1280
Gastric juice					
Normal...............	3	—	—	39	111
Normal...............	6	—	—	28	770

total extracellular diluting fluid of the organism as shown by Griffiths and Maegraith[23] and others. The volume into which the ^{24}Na is distributed (in per cent of body weight) gives a value of 23–25 in the rabbit,[17,18,20,23] and a similar value was obtained from an experiment in which the dilution of ^{38}Cl was determined.[17,24] In rats the value 23–29 is found.[18,25] The extracellular fluid in human subjects constitutes 26.5% of the body weight, with extreme values of 23 and 29%. The interchange equilibrium is reached after 3 hours or less; in patients with congestive heart disease 6 to 12 hours are required.[19]

E. APPARENT EXTRACELLULAR VOLUME

The intracellular nature of elements like potassium and phosphorus is strikingly shown by the extent of dilution observed when labeled potassium and phosphorus are introduced into the circulation.

When calculating the diluting volume of the rabbit from the percentages of the radioactive isotopes penetrating the organs shortly after injection into the circulation,[2] we find the diluting volumes for potas-

[23] J. H. E. Griffiths and B. G. Maegraith, Nature, 143, 159 (1939). J. F. Manery and W. F. Bale, Am. J. Physiol., 126, 578 (1939).

[24] J. F. Manery and L. F. Haege, Am. J. Physiol., 134, 83 (1941).

[25] D. M. Greenberg, W. W. Campbell, and M. Murayama, J. Biol. Chem., 136, 35 (1940).

sium and phosphorus to be 70 and 33%, respectively, of the body weight 5 minutes after the injections (see Tables 61 and 63, pages 196 and 197); the corresponding figure for sodium and chloride amounts to only 15%. Still larger differences are obtained when the experiment is continued. The high values clearly indicate cellular penetration by ^{42}K and ^{32}P (compare page 234).

VI. Determination of Circulation Time

Radiosodium has been applied in studies of circulation time in patients with peripheral vascular disease. A labeled sodium chloride solution is injected into a vein and the time required to reach a foot is determined by placing a counting tube at the extremity and timing the interval between injection and audible registration by the counter. The average value for the circulation time was found to be 45 seconds, the values varying between 15 and 90 seconds.[26] Since there is a constant interchange of sodium between plasma and extravascular fluid, the amount of radiosodium in the foot will increase until equilibrium is established between these two. The manner in which equilibrium is built up depends on the volume traversing the region per unit time, and on the relation between intracellular and vascular spaces. The times for the attainment of equilibrium were found to be different in normal subjects and in cases of peripheral vascular disease.[26,27]

In normal subjects the time taken by the blood to flow between the beginning of the femoral artery and the end of the tibial artery was found to be 22 seconds.[26] The velocity of the blood flow in infants and children has been measured by determining the time between the injection of radiosodium into one arm and its arrival in the opposite hand. The latter was signaled by a Geiger counter. By this method, the average time in 22 children between 2 and 12 years was found to be 11 seconds, with a range of 5 to 17 seconds. The time in 14 infants between 6 weeks and 22 months of age averaged 7 seconds with a range of 3 to 12 seconds[28] (see also Hamilton and Stone[29]).

The figure found for normal adults at rest was 17 seconds, while after exercise the figure was reduced to 13 seconds. The circulation

[26] E. H. Quimby and B. C. Smith, *Science*, **100**, 175 (1944).

[27] B. C. Smith and E. H. Quimby, *Surg. Gynecol. Obstet.*, **79**, 142 (1944); *Radiology*, **45**, 335 (1945).

[28] G. Sohrne, *Acta Radiol.*, **26**, 279 (1945).

[29] J. G. Hamilton and R. S. Stone, *Radiology*, **28**, 178 (1937).

rate decreased to 23 seconds with increased intrathoracic pressure (deep breath, glottis closed). Radiosodium with an activity of 2 to 5 microcuries per kilogram body weight, dissolved in 0.1 to 0.5 ml. water, was injected in these experiments.[30]

In numerous cases of peripheral vascular disease the circulation time of blood was determined by using radiosodium as a tracer.[27] Circulation times obtained in normal human subjects and in patients having peripheral vascular disease are given in Table 73.

TABLE 73

Circulation Times, Arm-to-Foot[27]

Diagnosis	Number of cases	Average time, sec.	Range, sec.
Arteriosclerosis	24	45	20–105
Arteriosclerosis with diabetes	13	42	25–90
Arteriosclerosis with diabetes and infection	5	27	20–40
Thromboangiitis obliterans	12	33	20–70
Scleroderma	5	44	15–75
Chronic varicose ulcer	3	38	30–45
Hypertension	13	41	20–80
Post-frozen feet	6	43	25–60
Miscellaneous	39	34	15–75
Normal (no evident vascular disorder)	11	43	20–55
All cases	131	39	15–105

The determination of the circulation time was found to be of special value in determining the advisability of continuing conservative treatment instead of more radical local surgery, and particularly in determining the site of amputation at which healing might be expected.[27]

That circulation in the extremities is somewhat lowered in patients suffering from rheumatoid arthritis was shown by comparing the rate of distribution of radiosodium into the blood stream of patients suffering from this disease with the rate of distribution in healthy human subjects. The average percentage distribution figures were 72 and 63, respectively.[31]

[30] J. P. Hubbard, W. N. Preston, and R. A. Ross, *J. Clin. Invest.*, **21**, 613 (1942).
[31] C. A. Tobias and J. J. Bertrand, *personal communication.*

Measurements of the circulation time were previously carried out with natural radioactive bodies. Blumgart and Weiss[32] injected an active deposit of radium into the antecubital vein of one arm, detecting the arrival of the radium in the other arm with a properly shielded cloud chamber. The circulation time of the red corpuscles is discussed in Chapter XI.

VII. Application of Radiosodium in the Study of Pulmonary Resuscitative Procedures

Thompson et al.[32a] describe a method of determining the effect of pulmonary resuscitative procedures on the circulation by the use of radioactive sodium.

^{24}Na, in normal saline, injected into the circulatory system of the living animals, is rapidly carried throughout the body; its presence in any region can be demonstrated by placing the counter against the surface of the body at that part. In an animal whose circulation has ceased, such distribution could not occur and a counter placed over the body at a place distant from the site of injection would register only a small background count, due to the presence of the sodium in the distant region. As long as the radioactive material remained essentially at this position, the count would remain the same. If, however, any movement of the blood in the veins and arteries could be brought about by a resuscitative procedure, the radioactive sodium would also be moved, and any appreciable shift toward the counter would be demonstrated by an increase in the counting rate.

By this method it has been possible to demonstrate that alternate mechanical inflation and deflation of the lungs, or either operation alone, produce a movement of the blood within the vascular system. This movement is sufficient in the dead but heparinized animal to circulate some of the blood throughout the entire body. Mechanical resuscitators, using alternating positive and negative pressures, produce the greatest circulation. As soon as post-mortem clotting occurs, little or no motion of the blood can be brought about by resuscitative procedures. When intravenous heparin is used to prevent this clotting, the period during which blood can be moved is greatly prolonged.

[32] H. L. Blumgart and S. Weiss, *J. Clin. Invest.*, **4**, 15 (1927).

[32a] S. A. Thompson, E. H. Quimby, and B. C. Smith, *Surg. Gynecol. Obstet.*, **83**, 387 (1946).

VIII. Permeability of the Stomach Wall

The transfer of ions across the stomach wall has also been studied by means of radioactive tracers.[33] Isotonic solutions containing radioactive isotopes of chloride, sodium, and potassium ions were introduced into the stomachs of rabbits previously ligated at the pylorus. In a parallel series of observations, solutions were placed in an isolated gastric pouch of the stomach of a dog. Chloride, sodium, and potassium ions placed within the stomach were all transferred to some extent across the stomach wall into the general circulation. After one hour, however, the transfer was too slight to permit an approach to equilibrium. On the other hand, equilibrium between intestinal contents and serum was very nearly attained within the same time in control experiments in which the pylorus was not obstructed. The transfer of sodium and potassium ions was much slower than that of chloride.

IX. Movement of Water and Ions between Intestine and Blood

The rate at which heavy water leaves the gut is found to be strongly influenced by the salt concentration. The rate of migration with a one-third isotonic solution is more than twice that found with hypertonic solutions.

The rate of labeled chloride movement from gut to blood increases with increase in chloride concentration, but not in linear proportion. The reverse movement is independent of the gut chloride concentration.

The rate of sodium movement from gut to blood is greater from an isotonic chloride than an isotonic sulfate solution, although the gut sodium concentration is higher in the latter case.

Visscher and associates[34] found furthermore that the experimentally derived ratios between total directional rates and net transport rates differ as much as 200-fold from the ratios predicted on the assumption that movement is by diffusion. In certain instances, moreover, the direction of movement is the reverse of that predicted. These and further observations are in harmony with the hypothesis that there is a forced flow of fluid across the intestinal epithelium in both directions simultaneously and that differences in the solute content of the water

[33] O. Cope, W. E. Cohn, and A. G. Brenizer, Jr., *J. Clin. Invest.*, **22**, 103 (1943).

[34] M. B. Visscher, R. H. Varco, Ch. W. Carr, R. B. Dean, and D. Erickson, *Am. J. Physiol.*, **141**, 488 (1944). M. B. Visscher, E. S. Fetcher, Ch. W. Carr, H. P. Gregor, M. S. Bushey, and D. E. Barker, *ibid.*, **142**, 550 (1944).

in the two streams and the relative rates of the streams determine the direction and magnitude of the net transport. The total turnover rate of sodium between the intestine and the blood of the dog is approximately equal to the total plasma sodium per 83 min.

X. Permeability of the Blood — Cerebrospinal Fluid Barrier

The exchange of ions between blood and cerebrospinal fluid is found to be very slow as compared with that between blood and intestinal fluid. Many hours are required to reach the distribution ratios normally found between spinal fluid and blood plasma. Of the ions investigated, potassium showed the highest rate of permeation. In the dog the rates of increase in concentration of the labeled ions in the cerebrospinal fluid follow the order: $K > Na > Br > Rb > Sr > P > I$.

Following their injection into the blood stream, the radioactive potassium, rubidium, and phosphorus contents of the cerebrospinal fluid exhibit pronounced maxima at about 20, 30, and 60 min., respectively, while sodium exhibits no maximum.

Evidence has been produced in these investigations that the exchange between blood and brain takes place by a process of secretion.[35]

XI. Penetration into the Aqueous Humor

In the anesthetized dog, ^{24}Na concentration in the aqueous humor reaches about 75% of the plasma level 45 minutes after intravenous injection of the isotope. The rate of approach to equilibrium between plasma and a cerebrospinal fluid sample from the cisterna magna is 25 to 45% as great as in the case of the aqueous humor.[36] In the rabbit it was found that sodium and chloride enter the anterior chamber of the eye from the blood stream at rates sufficient to produce 50% of the concentration present in the plasma after approximately 40 minutes. The rate of accumulation of ^{32}P in the anterior chamber is much less rapid.[36a]

In the study of the mode of entrance of sodium into the aqueous humor radiosodium was administered intraperitoneally to rabbits with

[35] D. M. Greenberg, R. B. Aird, M. D. D. Boelter, W. W. Campbell, W. E. Cohn, and M. M. Murayama, *J. Biol. Chem.*, **140**, ic (1941); *Am. J. Physiol.*, **140**, 47 (1943–44).

[36] M. B. Visscher and C. W. Carr, *Am. J. Physiol.*, **142**, 27 (1944).

[36a] V. E. Kinsey, W. M. Grant, D. G. Cogan, J. J. Livingood, and B. R. Curtis, *Arch. Ophthalmol.* Chicago, **27**, 1126 (1942).

the common carotid of one side occluded. Aqueous samples were taken from both eyes 40–60 minutes after the injection. At this time, about 50% of the activity level of the plasma was reached in the aqueous humor of both eyes and there was no significant difference between the two sides. Occlusion of the common carotid reduces the mean blood pressure in the ipsilateral ophthalmic arteries by at least 30 mm. mercury. This would cause a large decrease in the rate of exchange of any aqueous constituent entering the eye by ultrafiltration. The experiment thus proves that sodium enters by secretion.[37]

XII. Permeability of the Placenta

Radiosodium was injected by Pohl and Flexner[38] into the tail vein of a rat and, after a suitable time interval, the fetuses were delivered by Caesarean section and a sample of heart blood was taken from the mother. The radioactivity of 1 g. fetus was then compared with that of 1 ml. maternal plasma. The rat fetus was found to come within 10% of equilibrium with the ^{24}Na in the maternal plasma only after 6 hours, while the corresponding figure for the fetus of the cat was 12 to 18 hours. This result contrasts strikingly with the case of the maternal extracellular fluid, which comes to within 10% of equilibrium with intravenously injected ^{24}Na in about 4 minutes.

There is a large increase with increase of gestation age in the transfer rate of ^{24}Na across a unit weight of the rat's placenta. From a gestation age of 14 days until term, the transfer rate of ^{24}Na per gram of placenta increases 6 times. During the last third of pregnancy, about 27 times as much sodium passes across the placenta as is incorporated in the growing tissues.[39]

The placentas of guinea pigs[40] and rats belong to the hemochorial group. Both have transfer rates per gram of placenta of the same magnitude at comparable stages of pregnancy.

The permeability of the endotheliochorial placenta of the cat[41] to sodium was found to be low, since the cat fetus comes within 10% of equilibrium with ^{24}Na in the maternal plasma only after 12–18 hours. The fetus receives across the placenta an average of about 25 times as much ^{24}Na as is incorporated in the growing tissue.[38]

[37] E. Barany, *Nature*, **157**, 770 (1946); *Acta Physiol. Scand.*, **13**, 47 (1947).

[38] H. A. Pohl and L. B. Flexner, *J. Cellular Comp. Physiol.*, **18**, 49 (1941).

[39] L. B. Flexner and R. B. Roberts, *Am. J. Physiol.*, **128**, 154 (1939).

[40] L. B. Flexner and H. A. Pohl, *Am. J. Physiol.*, **132**, 594 (1941).

[41] H. A. Pohl and L. B. Flexner, *J. Biol. Chem.*, **139**, 163 (1941).

The rates of placental transfer of ^{24}Na in the rabbit,[42] the goat,[43] and the sow[44] were determined at various stages of the gestation period. The rate of transfer of sodium per gram placenta was found to increase in each animal as gestation proceeds until just before term, at which time there is a sharp decrease.

In human placenta the rate of transfer of sodium per unit weight increases markedly as gestation proceeds. At a gestation age of 10 weeks a sodium transfer of 0.76 mg. per gram placenta per hour was observed; at a gestation age of 37 weeks a value about six times as large was found.[45]

The above investigations show that the rates of transfer depend upon the morphological structure of the placenta: the smaller the number of tissue layers placed between maternal and fetal circulation, the greater the rate of transfer across a unit weight of placenta. A correlation was found, furthermore, between the supply of sodium transferred to a unit weight of fetus and the rate at which that unit weight of fetus was growing.[46]

By making use of diluted heavy water as a tracer the rate of passage of water through the placenta can be measured. In the course of 30 minutes 75% equilibrium with the maternal blood water of the guinea pig is attained. A 1-g. fetus receives across the placenta about 150 times as much water, and a 10-g. fetus 500 times as much water, as is incorporated in the growing tissue.[47] Inorganic phosphate reaches the fetus in an amount approximately equal to the phosphorus retained in growth.[45]

Radioiron, when fed in single doses near termination of pregnancy, appears rapidly in the fetal circulation — measurable amounts being present in 40 min.[47a]

The rate at which water is delivered to the amniotic fluid is such that a volume of water equal to the volume of this fluid is exchanged, on the average, about once an hour at all periods of gestation in the

[42] L. B. Flexner and H. A. Pohl, *Am. J. Physiol.*, **134**, 344 (1941).

[43] H. A. Pohl, L. B. Flexner, and A. Gellhorn, *Am. J. Physiol.*, **134**, 338 (1941).

[44] A. Gellhorn, L. B. Flexner, and H. A. Pohl, *J. Cellular Comp. Physiol.*, **18**, 393 (1941).

[45] W. S. Wilde, D. B. Cowie, and L. B. Flexner, *Am. J. Physiol.*, **147**, 360 (1946).

[46] L. B. Flexner and A. Gellhorn, *Am. J. Obstet. Gynecol.*, **43**, 965 (1942).

[47] L. B. Flexner and A. Gellhorn, *Am. J. Physiol.*, **136**, 750 (1942).

[47a] W. T. Pommerenke, P. F. Hahn, W. F. Bale, and W. M. Balfour, *Am. J. Physiol.*, **137**, 161 (1942).

guinea pig. The rate of transfer of sodium to the amniotic fluid of the guinea pig is much lower than that of water. At various stages of gestation the rate of transfer of sodium is about 50 times lower than the water transfer.[48] Extremely small but definite amounts of radioactive cobalt injected or fed to pregnant cows were transmitted across the placenta and stored in the liver of the fetus (see page 181).

XIII. Absorption from the Vagina

Radiosodium found application in the study of absorption of sodium instilled into the vagina. The amount of salt instilled ranged from 105 to 150 mg., about 10 ml. of solution being used in each instance. Blood samples taken at different intervals were examined for their radioactivity. Wide fluctuations were observed in the amount of sodium absorbed — from 0.2 mg. after an interval of 30 minutes in a normal subject to 11.5 mg. after 18 hours in a patient 12 days post partum.[49]

XIV. Rate of Penetration of Potassium into Tissue Cells

Large amounts of potassium are present in muscle cells and cells of many other organs. Rabbit muscles contain about 430 milligram per cent potassium, and similar values are found for the muscles of other mammals. Of the 430 milligram per cent potassium of the muscle, about 428 mg. is to be found in the cells and only 2 mg. in the interspaces. Similar figures are found for rats and frogs. The plasma and presumably also the extracellular fluid of the rabbit contain only about 18 milligram per cent potassium, similar values being found for most other mammals.

In order to investigate whether exchange takes place between cellular and extracellular potassium ions, labeled potassium was administered to rats,[50,51] rabbits,[51,52] and frogs,[53,54] and the distributions of

[48] L. B. Flexner and A. Gellhorn, *Am. J. Physiol.*, **136**, 757 (1942).

[49] W. T. Pommerenke and P. F. Hahn, *Am. J. Obstet. Gynecol.*, **46**, 853 (1943).

[50] M. Joseph, W. E. Cohn and D. M. Greenberg, *J. Biol. Chem.*, **128**, 673 (1939).

[51] W. O. Fenn, T. R. Noonan, L. J. Mullins, and L. Haege, *Am. J. Physiol.*, **135**, 149 (1941/42).

[52] G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab Biol. Medd.* **16**, No. 1 (1941).

[53] T. R. Noonan, W. O. Fenn, and L. Haege, *Am. J. Physiol.*, **132**, 474 and 614 (1941).

[54] G. Hevesy, L. Hahn, and O. Rebbe, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **16**, No. 8 (1941).

the ^{42}K ions between plasma potassium and tissue potassium were compared after varying periods of time. Such an exchange would obviously necessitate passage through the cell wall. The measurements were carried out by comparing the radioactivity of 1 g. plasma and 1 g. muscle tissue. If the ^{42}K ions were prevented from penetrating the muscle cells, the extracellular volume of the muscle would contain all the ^{42}K present in the tissue and, assuming this volume to represent 11% of the muscle weight, 1 g. muscle would only be one-ninth as active as 1 g. plasma. On the other hand, if the ^{42}K ions rapidly penetrated the muscle cells, a proportional partition of ^{42}K between the potassium of the plasma and that of the muscle would soon take place and we should find 1 g. muscle to be 22 (= 430/20) times as active as 1 g. plasma.

The partition of ^{42}K between plasma and muscle cells can be determined by comparing activity of 1 g. plasma with that of 1 g. muscle. However, there is also another mode of approach which does not involve the investigation of the activity of the muscle tissue. This second method of determination of the extent of cellular exchange is based upon the measurement of loss of ^{42}K from circulating blood. This loss is due mainly to penetration of ^{42}K into the tissue cells and it is therefore possible to estimate from the decrease in the ^{42}K content of the plasma the extent of ^{42}K penetration into the cells. If the potassium contents of the plasma and the cells are known, we can calculate the percentage replacement of cellular potassium by plasma potassium which takes place in the course of the experiment. This "disappearance" method is mainly of interest in the investigation of potassium interchange between cells and intercellular space of human subjects. In these investigations, the direct method based upon comparison of the ^{42}K contents of plasma and tissue samples is obviously inapplicable.

An exhaustive investigation of the interchange rate of plasma potassium and potassium present in the organs of the rabbit, guinea pig, rat, and frog was made by Fenn and colleagues (Figs. 45–47).[51,53]

As seen from the figures, for the liver, diaphragm, heart, lung, and skin, the specific activity values of the tissue potassium are higher than the values for plasma potassium (taken as 1). The ratio of the specific activities of the organ and plasma potassium lay above 1, indicating a percentage interchange of over 100%. In the case of the kidney, 1 mg. potassium contains 2.5 times as much ^{42}K as 1 mg. plasma.

Lower interchange values found by others[52,55] are interpreted by Fenn *et al.* as due to slight traces of radiosodium in the samples used.
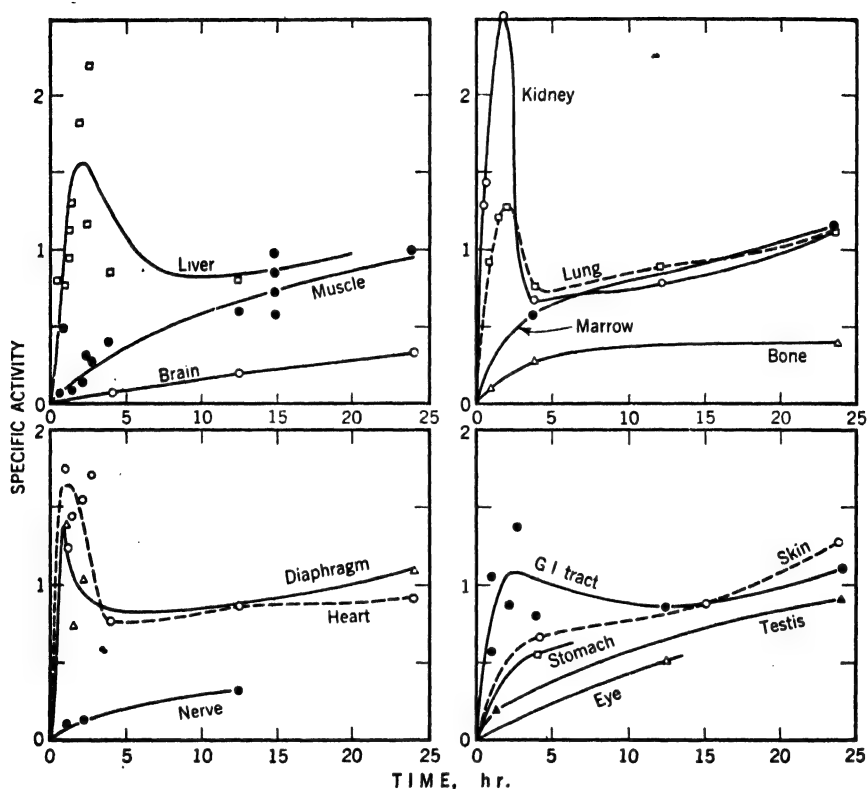
Fig. 45.   Specific activity of potassium in various organs relative to the specific activity of plasma potassium ($=1$).[51]

In all cases the final activity of the plasma is compared with that of the tissue.   Actually the average plasma activity during the experiment should be considered.   Thus, in the event of a complete interchange, the activity of 1 mg. tissue potassium should be higher than that of 1 mg. plasma potassium.

This fact, however, can hardly be responsible for the 250% potassium exchange found, for example, in the case of the kidneys.   We have to interpret this figure as indicating not only an interchange between the potassium of the kidney and that of the plasma, but an accumulation of a part of the injected labeled potassium in the kidneys;

[55] W. E. Cohn, *Am. J. Physiol.*, **133**, 242 (1941).

the same applies to the figures obtained for other viscera. Two hours after injecting ^{42}K into the dorsal lymph sac of the frog the potassium of the skin was found to show as much as 320% interchange. A possible explanation of these observations is the following. In the above investigation in which an appreciable amount of labeled potassium chloride, namely 0.78 milli-equivalent per kilogram rabbit weight, was administered, the organism reacted to the increase in normal potassium content of the plasma by a swift removal of a substantial part of the additional potassium by the liver and other viscera. The correctness of this explanation could be tested by comparing the percentage uptake of ^{42}K by the kidneys, for example, after administration of labeled potassium of negligible weight and after injecting a large amount of potassium containing ^{42}K. The problem of ion permeability to muscle is discussed by Krogh[56] and Buchthal[57] in recent reviews (cf. also Chapter XI).
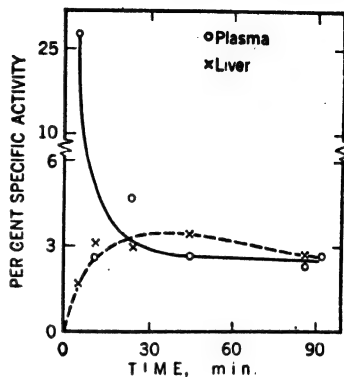
Fig. 46. Change in ^{42}K content of plasma and liver of the rabbit with time.[51]
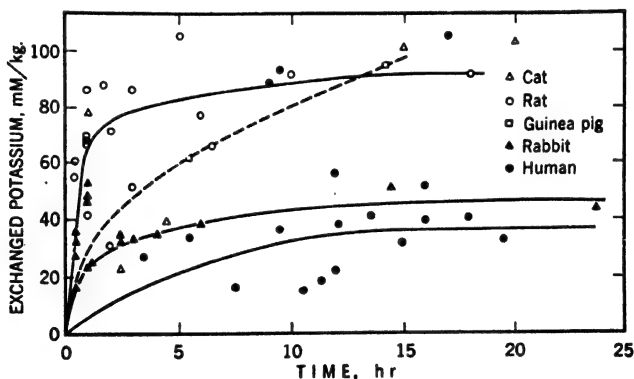
Fig. 47. Interchange between tissue and plasma potassium at various times after ^{42}K administration.[51] Total body potassium is usually about 80 millimoles per kilogram.

[56] A. Krogh, *Proc. Roy. Soc.*, London, **B133**, 195 (1946).
[57] F. Buchthal, *Ann. Rev. Physiol.*, **9**, 119 (1947).

That a large part of ^{42}K taken up by the liver is soon returned to the circulation is shown in Figure 46. In spite of the swift removal of ^{42}K from the plasma, due to entrance into the organ, the specific activity of the liver potassium is again down at the plasma potassium activity level after 90 minutes. Brain and bone tissue show the lowest ^{42}K contents. The low rate of penetration of ions into the brain tissues is a general phenomenon. The low interchange value for bone indicates that the greater part of the potassium is present inside the apatite crystals and is not accessible to interchange in the course of 1 or 2 days (see page 222).

To what extent interchange between the total potassium content of the body occurs in the course of 1 day was investigated by dissolving the animal and by comparing the ^{42}K content of 1 mg. body potassium with the corresponding plasma value. For total interchange, the ^{42}K content (per milligram potassium) should be the same in each case. As is seen in Figure 47 total interchange is obtained in the rat, but not in the rabbit. The ordinate values indicate exchanged potassium in millimoles per kilogram. The potassium content of the rabbit was found to be 82.9 millimoles per kilogram, only half the rabbit's potassium exchanging in the course of 1 day. The path of the curve indicates that the other half of the potassium content of the rabbit does not interchange or interchanges only at a much slower rate.

In the case of human subjects the following procedure was used.[51] The subject drank radioactive potassium chloride; urine samples were then collected at intervals. It is assumed for purposes of calculation that the isotope composition of the urine is the same as that of the plasma. If the urine is analyzed for both potassium and radioactivity it is possible to calculate the specific activity of the plasma potassium. In some cases, in addition to the potassium content and radioactivity of the plasma, those of the saliva were determined as well. Plasma and urine activity per mg. potassium were found in an individual to be 1.79 and 1.77, respectively. Assuming the potassium content per kilogram of the human body to be about equal to that of the animals analyzed (70 millimoles per kilogram for the rat, 83 millimoles per kilogram for the rabbit, and 77.6 millimoles per kilogram for the cat), we can calculate from the plasma activity figures obtained at different times the extent of potassium interchange in the human body at the corresponding dates. As seen from Figure 47, about half the body potassium interchanged in the course of a single day. In this case as in that of

the rabbit half the body potassium does not exchange at all or only at a much slower rate. Since a complete interchange of muscle potassium and plasma potassium was found to take place, according to Fenn and co-workers, and since two-thirds of the rabbit's potassium is located in the muscles, the last-mentioned result still awaits explanation.

The amount of interchangeable potassium in the human body was also calculated from the urine figures (potassium content and radioactivity) alone.[58] The results obtained indicate an interchange of a large part of the body potassium in the course of 1 day.

Denervation of the gastrocnemius muscle of rats was found to facilitate penetration of ^{42}K 2–9 minutes after intraperitoneal injection of radiopotassium. The ^{42}K content of the denervated muscles was found to be 2.76 times that of the controls.[59]

In their studies on the exchange of potassium between frog heart muscle and the bathing fluid, Krogh and associates[60] made use of labeled potassium. Cohn and Brues,[61] using chick embryo muscle cultures, determined that the amount of potassium penetrating these cells was 14.0×10^{-7} millimole per square centimeter per hour. For the amount of phosphorus they obtained an appreciably lower figure, 1.57×10^{-7}.

The permeability of the red corpuscles to potassium is discussed in Chapter XI.

XV. Effect of Muscular Exercise on Potassium Interchange

In order to investigate the effect of muscular exercise on rate of interchange of potassium between muscle cells and plasma, Hahn and Hevesy[62] administered labeled potassium to six rats by subcutaneous injection (3.1 mg. of potassium to each). Three rats were forced to perform heavy muscular work, swimming for 30 minutes; three were kept as controls. The swimming started 12 minutes after administration of ^{42}K. Two rats were almost exhausted by the intense swimming and by their strenuous efforts to escape from the spacious swimming pool; the third rat (VI) had to be assisted in the last phase of the swimming. All the rats were killed as soon as the swimming was terminated.

[58] G. Hevesy, *Acta Physiol. Scand.*, **3**, 123 (1942).

[59] C. P. Lyman, *Am. J. Physiol.*, **137**, 392 (1942).

[60] A. Krogh, A. L. Lindberg, and B. Schmidt-Nielsen, *Acta Physiol. Scand.*, **7**, 221 (1944).

[61] W. E. Cohn and A. M. Brues, *J. Gen. Physiol.*, **28**, 449 (1945).

[62] L. Hahn and G. Hevesy, *Acta Physiol. Scand.*, **2**, 51 (1941)

The ^{42}K contents per gram of gastrocnemius muscle of the resting and the swimming rats are seen in Table 74.

TABLE 74

Uptake of ^{42}K by Gastrocnemius Muscle of Resting and Swimming Rats[62]

Rat	Time after ^{42}K administration, min.	^{42}K per gram muscle (relative figures)
Resting I..................	48	1.0
II..................	49	1.3
III..................	51	1.3
Swimming IV..................	47	5.4
V..................	48.5	3.3
VI..................	51	6.5

As seen from the figures in the table, about four times as much ^{42}K penetrates the working muscles as the resting ones during the same period. In spite of the fact that the muscle, as found by numerous investigators, loses some of its potassium content during work, a much larger percentage of the potassium atoms which were in the plasma (extracellular space) at the start of the experiments is found in the working than in the resting muscle. Similar results were obtained by Noonan et al.[63] by administering labeled potassium intraperitoneally to rats in which one muscle was stimulated (Table 75).

TABLE 75

Uptake of ^{42}K by Stimulated and Nonstimulated Muscles of the Rat[63]

Muscle	Time, hr.	Ratio of ^{42}K uptake by stimulated and control muscle
Gastrocnemius and soleus..............	1.3	1.6
Tibialis and peroneus..................		2.7
Gastrocnemius and soleus..............	1.5	3.8
Tibialis and peroneus..................		4.4
Gastrocnemius and soleus..............	1.0	3
Tibialis and peroneus..................		6

[63] R. T. Noonan, W. O. Fenn, and L. Haege, *Am. J. Physiol.*, **132**, 612 (1941)

While, in the first-mentioned experiment, stimulation increased the [42]K uptake 4.2 times on the average, in the second experiment an increase of 3.6 times was found to take place. Denervation also was found to increase rate of penetration of [42]K into muscles, but this increase was only about twofold.

In experiments with frogs, only a slightly greater fraction of the potassium content of the muscles was found by Noonan and colleagues[63] to interchange in the stimulated muscle. In isolated frog muscles stimulated in Ringer solution containing radioactive potassium, no increased penetration could be observed.

The increased uptake of [42]K by the stimulated muscle is interpreted[63] as due to enhanced circulation. The possibility that we are concerned in the stimulated muscle with an increased rate of interchange between intra- and extracellular potassium cannot, however, be excluded.

XVI. Rate of Penetration of Sodium into Tissue Cells

While most of the potassium content of the muscle tissues is contained in the cells, only a minor and unknown part of the sodium content is to be found in the cellular space. This being the case, we cannot determine the rate at which labeled sodium diffuses into cells of the muscles and other tissues. However, it is possible to accumulate appreciable amounts of sodium in muscle cells of rats when the animals are kept on a prolonged potassium-free diet. The low potassium content of plasma, which is found to be the result of a potassium-free diet, leads to a loss of potassium from muscle cells into plasma, the loss of potassium by muscle cells being to some extent compensated by an uptake of sodium. Up to two-thirds of muscle sodium is found to be intracellular. Heppel[64] administered labeled sodium to rats kept for a considerable time on a potassium-free diet, and found a fairly rapid entrance of [24]Na into cells, a proportional partition of [24]Na between plasma sodium and muscle sodium being obtained in the course of 60 minutes, as seen in Table 76.

Similar results were obtained by Ussing,[64a] who used isolated frog sartorii. These were first allowed to take up [24]Na and were then placed in an inactive Ringer solution, the solution being renewed at intervals. It was found that the sodium content of the fibers was renewed in 30 minutes. This rate was unaffected even when the potassium content

[64] L. A. Heppel, *Am. J. Physiol.*, **128**, 449 (1940)
[64a] H. H. Ussing, *Nature*, **160**, 262 (1947).

of the solution was increased to 10 times its normal value. It is of interest to ask if this penetration is necessarily an active process. Ussing shows this to be very unlikely. He calculates that the energy necessary to perform a corresponding active transport is about 0.15 cal. per gram of fiber per hour. Such an energy can hardly be supplied to obtain sodium transport since the total energy output in 1 gram of resting muscle amounts to no more than the above-mentioned figure.

TABLE 76

Penetration of ^{24}Na into Muscles of Potassium-Deprived Rats[64]

Rat weight, g.	Days on diet	Time after ^{24}Na given, min.	Ratio of ^{24}Na per unit wt. muscle to ^{24}Na per unit wt. serum	Ratio of total Na per unit weight muscle to total Na per unit wt. serum
117	40	5	14	39.2
101	36	10	17	23.5
90	34	10	15	26.8
102	40	20	23	36.4
117	37	31	25	34.4
80	37	60	31	32.7
98	44	60	33	35.0
108	34	182	28	31.1
110	44	187	38	41.2
101	42	215	32	30.2
99	34	260	33	32.6

Krogh[56] calculates from the above figures the remarkably high value of 4.6×10^{-3} cm. per hour for the penetration coefficient of sodium into muscle cells. The significance of the coefficient of penetration is discussed in Chapter XI.

The rate of penetration of sodium and rubidium ions through the excised skin of the frog was measured using radioactive isotopes as tracers for the salt which had passed through. Diffusion was measured for two-hour periods between an isotonic salt solution bathing the inner side of the skin and distilled water on the outside. The rate of passage for the pure salt was: 4.7×10^{-12} mole cm.$^{-2}$ sec.$^{-1}$ for sodium chloride and 125×10^{-12} mole cm.$^{-2}$ sec.$^{-1}$ for rubidium chloride.[65]

[65] L. J. Katzin, *Biol. Bull.*, **77**, 302 (1939).

XVII. Study of the Influence of the Neurohypophyseal Principles on the Sodium Metabolism in the Axolotl

Many fresh water animals have the ability to take up certain ions, especially Cl^- and Na^+, from the surrounding medium.[56] The uptake may take place through the gills (fish), through special organs (mosquito larvae), or through the whole surface (frog). In species where this mechanism of salt uptake is well developed (frog, goldfish) ions may be taken up even from 0.01 millimolar solution, the salt thus being concentrated about 10,000 times on the passage from the medium into the blood of the animal.

The mechanism of this "active" uptake of sodium and chlorine was studied by Barker Jörgensen et al.[65a] As experimental animals full grown axolotl (*Amblystoma mexicanum*) weighing between 65 and 103 g. were used. During the experiment the animals were kept in dilute labeled solutions 0.001 to 0.003 normal with respect to sodium. The concentration of these ions in the blood of the animals being about 0.1 N, the amount of labeled Na^+ and Cl^- taken up is interpreted to be solely due to an "active" uptake, and the uptake by "normal" diffusion to be negligible. The activity of the solution in which the axolotl was kept was not allowed to decrease below 50%, and this permitted neglecting the loss of $^{24}Na^+$ or $^{38}Cl^-$ by the axolotl due to diffusion into the outer solution or with the urine. The active uptake of ions was calculated in the following way:

The rate of active uptake, *i.e.*, the amount of the ion in question which passes into the animal per unit time is di/dt, whereas the rate of decrease of the activity is dy/dt. Since the ion-absorbing cells cannot distinguish between active and inactive ions, we have:

$$\frac{-dy/dt}{di/dt} = y/A \qquad (a)$$

or

$$\frac{-dy/dt}{y} = \frac{di/dt}{A} \qquad (b)$$

If di/dt, the rate of active uptake, is taken as a constant during the

[65a] C. Barker Jörgensen, H. Levi, and H. Ussing, *Acta Physiol. Scand.*, **12**, 350 (1946). H. Ussing, C. Barker Jörgensen, and H. Levi, *Bull. soc. chim. biol.*, **29**, 280 (1947).

experimental period, we may replace di/dt by the constant U and thus obtain:

$$dy/dt = -(U/A)\,y \qquad (c)$$

The considered cases are: (I) A = constant; and (II) A is increasing or decreasing at a constant rate. For A = constant:

$$y = y_0 e^{-(U/A)t} \quad \text{or} \quad U = -\left(\frac{A}{t \log e}\right) \log \frac{y}{y_0} \qquad (d)$$

Insertion of the known values of y, y_0, A, and t gives the rate of active uptake U.

If a net uptake or excretion of the ion has occurred, we must subdivide the experiment in periods during which this net change proceeds at an approximately constant rate, B, where B is positive when the quantity of the ion in the medium increases:

$$A = A_0 + Bt \qquad (e)$$

Inserting equation e in c, we obtain:

$$dy/dt = -y\,\frac{U}{A_0 + Bt} \qquad (f)$$

This differential equation can be solved as:

$$y = e^{U/B \ln (A_0 - Bt) + K} \qquad (g)$$

where K is an integration constant, or:

$$y = e^K (A_0 - Bt)^{U/B} \qquad (h)$$

Inserting the values for the time, t, and the starting moment (designated y_0 and A_0), we obtain:

$$\frac{y_t}{y_0} = \frac{e^K (A_0 - Bt)^{U/B}}{e^K A_0^{U/B}} = \frac{A_t^{U/B}}{A_0^{U/B}} \qquad (i)$$

From i we obtain:

$$U = B\,\frac{\log (y_0/y_t)}{\log (A_0/A_t)} \qquad (j)$$

directly leading to the rate of active uptake, U.

The amount of sodium taken up actively and excreted in the course of 24 hours is shown in Table 77. The table also shows the effect of

insipidin, a hypophysis extract, on active uptake and excretion of sodium.

TABLE 77

Effect of Insipidin on Uptake and Excretion of Sodium[65a]

Exp. No.	Equiv. Na taken up in 24 hr.		Increase in Na uptake on insipidin injection, %	Equiv. Na excreted in 24 hr.		Increase in Na excretion on insipidin injection, %
	Before insipidin injection	After insipidin injection		Before insipidin injection	After insipidin injection	
I	99	275	178	153	251	64
II	55	191	247	107	95	−11
III	122	324	156	41	162	295
IV	79	205	160	57	78	37
V	66	174	164	47	133	183
VI	50	246	390	80	245	206
VII	61	173	173	98	173	77
Mean	76 μ	227 μ	200%	83 μ	162 μ	95%

Injection of the oxytocic principle represented by the preparation Pitupartin induced a rapid net loss of sodium and chlorine lasting for 2–5 days. This loss was found to be due to an increased excretion, whereas the active sodium uptake was practically unaffected.

Injection of 1 ml. 4% sodium chloride did not result in a significant uptake and excretion of sodium; the animals may even continue to gain sodium and chlorine after the injection. These experiments disprove the assumption that the concentration of sodium or chlorine ions in the body fluids determines the rate of active uptake through a direct action of ions on the transporting cells.

XVIII. Permeability of Yeast Cells to Potassium

The interchange of potassium between yeast and its nutrient solution was investigated by using radiopotassium (^{42}K) as an indicator.[66] In strongly fermenting yeast, almost complete interchange was found to take place between the potassium of the yeast and the potassium of the nutrient solution in the course of 2 hours. In most experiments in which fermentation was slight, much slower potassium exchange was observed.

[66] G. Hevesy and N. Nielsen, *Acta Physiol. Scand.*, **2**, 347 (1941).

In Table 78, data on the distribution of ^{42}K between yeast and its nutrient solution are given. In those cases in which nonlabeled yeast was placed in labeled nutrient solution, the ratio of the ^{42}K content of the yeast to that of the nutrient solution of equal volume is given. In the converse cases, the ratio of the ^{42}K content of the nutrient solution

TABLE 78

Distribution of ^{42}K between Yeast and Nutrient Solution[66]

^{42}K initially present in	Time, hr.	Distribution coefficient of ^{42}K between yeast and solution			Fermentation strength	Increase in dry substance of yeast, %
		Calculated for		Found		
		No K exchange[a]	Total K exchange			
Solution....	2	0 16	0.63	0.38	Slight	5.5
Yeast......	20	0.03	1.71	0.91	Very strong	3.9
	44	0	1.23	1.14	Very strong	1.0
Solution....	2	0.26	0.90	1.05	Very strong	11.3
Yeast	2	0.01	1.04	0.14	Fairly strong	−0.4
Solution....	2	0.16	0.61	0.14	Fairly strong	5.7
Yeast......	2	0	1.34	0.14	Very strong	1.1
Solution....	1.5	0.33	0.88	0.63	Very strong	4.3
	23	0.78	1.48	1.61	Very strong	56.5
Solution....	22	0.64	1.19	0.96	Very strong	166.8
	22	0.73	1.14	0.85	Very strong	174.5

[a] Assuming all ^{42}K found in the yeast cells is due to additional accumulation of labeled potassium in the cells.

to that of the yeast is given. In the third column of the table, values are shown for the distribution coefficient of ^{42}K which would be expected if only additional accumulation by the yeast, but no exchange of potassium between yeast and nutrient solution, took place. Due to the increase in the potassium content of the yeast during the fermentation, ^{42}K will *accumulate* in the yeast even in a total absence of potassium *exchange* between yeast and nutrient solution. If we denote the increment in the potassium content of the yeast by a and the original ^{42}K content of the nutrient solution by b, the ratio of the ^{42}K content of the yeast and the nutrient solution will work out to be $a/(b − a)$. In this calculation, the absence of potassium exchange between yeast and nutrient solution is assumed. In a similar way, the calculation is carried

out assuming total potassium exchange. In the fourth column are shown the distribution coefficients expected for complete interchange between yeast potassium and nutrient solution potassium. The figures are calculated from the data of the chemical analyses. In the case of full interchange, the ratio of ^{42}K and of total potassium content of the yeast and the nutrient solution must obviously be the same. In the fifth column are the values found for the distribution coefficient.

If no exchange took place, the values of the fifth column should correspond to the values in the third column; in the event of complete interchange, the former should correspond to the values of the fourth column. In partial interchange, the values of the fifth column should lie between the corresponding values of columns 3 and 4. The difference between the values of columns 4 and 5 will decrease with increasing interchange. Since, with increasing interchange, the ^{42}K content of the nutrient solution (or of the yeast) becomes more and more "diluted" by nonactive potassium moving in the opposite direction, we would underestimate the extent of the interchange in the case of a strongly pronounced interchange. In this case, the interchange is in fact markedly larger than indicated by the figures of column 5. Conway,[67] who used chemical methods, recently found that the membrane of yeast cells becomes more permeable during fermentation.

Radiopotassium was also applied in the study of the release of potassium by cells of bakers' yeast when fluoride is added to the nutrient solution.[68]

XIX. Effect of Irradiation on Permeability of Yeast Cells to Potassium

When investigating the effect of Röntgen rays on potassium permeability, yeast containing labeled potassium was shaken at room temperature with a nutritive solution containing 0.15 M potassium chloride. Part of the yeast was irradiated with 30,000 r. prior to this treatment, as described on page 243. The results obtained in these experiments are shown in Table 79.

Irradiation with the very substantial dose of 30,000 r. necessary to kill all but 10% of the yeast cells thus influenced the permeability of the cells to a slight extent only, the permeability of the irradiated cells being only 30% higher than that of the nonirradiated cells.

[67] E. J. Conway, *Biochem. J.*, **40**, 59 (1946).

[68] M. Malm, *Svenska Vetenskapsakad. Arkiv Kemi*, **A21**, No. 4 (1946); *ibid.*, **A25**, No. 1 (1947).

When the yeast cells were shaken with a nutrient solution containing arsenate, the amount of migrating ^{42}K was found to be reduced to about half of its normal value.

TABLE 79

^{42}K Migrating from Cells Irradiated with X-Rays and from Nonirradiated Cells into Nutrient Solution[69]

Sample	Time, hr.	Per cent ^{42}K migrated	Ratio of ^{42}K migrated from irradiated and nonirradiated cells
Control..............	2	8	1.3
Irradiated..........	2	10	
Control..............	2	11.8	1.2
Irradiated..........	2	13.8	
Control..............	4	22	1.4
Irradiated..........	4	30	

In contradistinction to Röntgen rays, an only partly lethal dose of ultraviolet radiation strongly influences the rate of passage of ^{32}P from the yeast cells to the nutrient solution (see page 244); this effect of ultraviolet radiation was not observed on the rate of migration of ^{42}K from the cells into the nutrient solution.

XX. Rate of Penetration of Bicarbonate into Tissue Cells

Rapid equilibrium of both intracellular and extracellular carbonate is attained; this is shown in experiments in which, soon after intravenous injection of radioactive bicarbonate, the specific activity of ^{11}C in the serum was found to be equal to that in the pancreatic juice, a secretion of intracellular origin.[70]

XXI. Rate of Penetration of Phosphate into Tissue Cells

The relatively slow exchange of ^{32}P between the inorganic phosphate of plasma and muscles in contrast with the much more rapid exchange between plasma and liver or kidney phosphate, has already been observed in early investigations. These early investigations also revealed a rapid incorporation of labeled phosphate into some of the

[69] G. Hevesy and K. Zerahn, *Acta Radiol.*, **27**, 157 (1946).

[70] E. G. Ball, H. F. Tucker, A. K. Solomon, and B. Vennesland, *J. Biol. Chem.*, **140**, 119 (1941).

organic phosphorus compounds present in the tissue cells of the muscles and other organs.[71, 73] When, after maintaining the plasma, containing 3 milligram per cent free inorganic P, at a constant activity level (1 μg. corresponding to 100 activity units), we find, after the lapse of 1 hour for example, that 1 g. muscle tissue contains 3000 activity units, we may conclude that 30 μg. phosphorus originally present in the plasma has penetrated into 1 g. muscle tissue. While an equal amount of phosphorus passes in the opposite direction, the amount of ^{32}P which moved from the tissue into the plasma during that time can be disregarded, in view of the high phosphorus content of muscle cells. In view of the low extracellular space of muscle tissue (see page 202), the activity to be found in the interspaces of 1 g. tissue can be calculated to amount to about 400 units only, 90% of the labeled phosphate of the muscle tissue being thus present in the cells.

Two hours after subcutaneous injection of labeled phosphate into the rat, 1 g. gastrocnemius tissue was found to contain twice as much labeled phosphorus as 1 g. plasma. Since the free phosphorus content of 1 g. plasma amounted to 50 μg., we can estimate that about 100 μg. penetrated the phase boundary of the muscle cells in the course of 2 hours. Very much higher figures are obtained, as seen in Table 80, for the liver, while lower figures are found for the brain.[72]

Rabbit muscle cells are less permeable than those of the rat, and very much lower figures were found for frog muscle. This difference is partly due to the lower temperature at which the permeability of the frog muscle cells was determined. At 22° C., in the course of 2 hours, 1.7 μg., and, at 0°, 20% of that amount was found to penetrate the muscle cells.[74] From the above data Krogh[75] calculates the coefficient of penetration of phosphate ion into frog muscles at 22° to be about 1.8×10^{-5} cm. per hour.

Furchgott and Shorr[76] measured the rate of penetration of labeled

[71] G. Hevesy and O. Rebbe, *Nature*, **141**, 1097 (1938). G. Hevesy, *J. Chem. Soc.*, **1939**, 1213. E. Lundsgaard, *Skand. Arch. Physiol.*, **80**, 291 (1938).

[72] G. Hevesy and H. v. Euler, *Svenska Vetenskapsakad. Arkiv Kemi*, **A15**, No. 15 (1942).

[73] J. Sacks, *Am. J. Physiol.*, **129**, 227 (1940).

[74] G. Hevesy and O. Rebbe, *Acta Physiol. Scand.*, **2**, 171 (1940).

[75] A. Krogh, *Proc. Roy. Soc. London*, **B133**, 195 (1946).

[76] R. F. Furchgott and E. Shorr, *J. Biol. Chem.*, **151**, 65 (1943); *Federation Proc.*, **1**, 2, 27 (1942); *ibid.*, **2**, 13 (1943). S. B. Barker, R. F. Furchgott, and E. Shorr, *Am. J. Physiol.*, **133**, 202 (1947).

phosphate into slices of cat cardiac muscle, incubated in bicarbonate–Ringer at 37.5°. They found that 1.7 μg. phosphorus penetrates 1 g. tissue per minute, this being about the amount found to penetrate the gastrocnemius muscle of the rat mentioned above. Penetration of phosphate at 20° was found to be only 20–10% of that measured at 37.5°.

TABLE 80

^{32}P Present Two Hours after Subcutaneous Administration to a 150-Gram Rat[72]

Organ	Per cent of administered ^{32}P present in 1 g. fresh tissue	^{32}P content per gram tissue as per cent of ^{32}P content of 1 g. plasma × 10⁻²
Plasma.................	0.062	
Liver.................	1.6	26
Sarcoma.............	0.92	15
Spleen...............	0.72	12
Muscle..............	0.12	1.9
Testes...............	0.15	2.4
Brain................	0.06	1.0

Table 81 shows permeability of the cell membrane of Jensen sarcoma tissue. As seen from the table, due regard was taken in the calculation of these data to the ^{32}P content of the extracellular fluid. The permeability (F) in mg. is given by $F = A(E/C)$ where A is the free inorganic phosphorus content of 1 g. plasma and C is the total ^{32}P content of 1 g. plasma. E, the total ^{32}P content of the cells of 1 g. sarcoma, is equal to $D - 0.25\ C$, where D = total ^{32}P content of 1 g.

TABLE 81

Amount of Phosphorus Passing Cell Membrane of Jensen Sarcoma[a] Tissue in Two Hours[72]

P content per gram plasma	^{32}P content per gram plasma	^{32}P content per gram sarcoma	^{32}P content per gram sarcoma cells	P passing cell membrane per gram sarcoma, mg.
A	C	D	E	F
0.046	100	1933	1908	0.87
0.041	100	1620	1595	0.65
0.065	100	1110	1075	0.65

[a] Rats with Jensen sarcoma weighed 150 g.

sarcoma tissue, and 0.25 = the extracellular volume of the sarcoma tissue. The final phosphorus content of the plasma was almost equal to the average value during the experiment.

Labeled phosphate, after passing the cell membrane, will speedily participate in phosphorylation processes and then enter various acid-soluble phosphorus compounds present in cells (see Chapter VIII). If the concentration of such compounds in the tissue cells is high while that of the inorganic cellular phosphate is low, the activity of the inorganic intracellular phosphorus in the tissue cells will be greatly diluted by this process. The specific activity of the inorganic cellular phosphorus is not, therefore, a strict measure of the permeability of the cell wall. However, some information regarding the permeability can also be gained by comparing the specific activity of the inorganic intracellular phosphorus with that of the inorganic extracellular (plasma) phosphorus. Such data are seen in Tables 82 and 83.

The permeability of liver-cells to phosphate was studied by Kalckar et al.[78] in experiments of very short duration in which the extracellular phosphate of the organs was removed by perfusion. The specific activity of the inorganic intracellular phosphorus of rabbit liver, 5 minutes after intravenous injection of labeled phosphate, was found to be 8–37% of that of the inorganic plasma phosphorus. In interpreting these figures we must take into account that the entering ^{32}P participates speedily in phosphorylation processes going on in liver cells (see Chapter VIII), while inactive inorganic phosphate ions are simultaneously split from organic phosphorus compounds. This process is instrumental in lowering the specific activity of the inorganic intracellular liver phosphorus. The above figures thus indicate a very high permeability of liver cells to phosphate.

The same authors carried out similar experiments with skeletal muscles. After the lapse of 15 to 20 minutes the specific activity of intracellular inorganic muscle phosphorus was found to be only 1.5% of the value for free plasma phosphorus. When the extracellular ^{32}P was not removed by perfusion of the tissue, the corresponding figure was 3.5. These results demonstrate the important role of the extracellular ^{32}P in the total ^{32}P of muscle tissue in experiments of very short duration. Kalckar and associates estimate that in the course of 1 minute, 1 μg. phosphorus penetrates (and leaves) the cells of 1 g. muscle tissue.

[78] H. M. Kalckar, J. Dehlinger, and A. Mehler, *J. Biol. Chem.*, **154**, 275 (1944).

Values for the specific activity of the inorganic phosphorus of rat liver at different times after subcutaneous injection[79] are seen in Table 84. The figures obtained after 0.5, 1, and 2 hours do not differ much and correspondingly the average specific activity during an experiment lasting 2 hours is only somewhat lower than that measured at the end of the experiment. This fact is important in calculating rate of renewal of phosphorus compounds present in the liver.

TABLE 82

Specific Activity of Inorganic Phosphate[a] Extracted from Rabbit Organs[76a]

Organ	Inorganic P, specific activity	Organ	Inorganic P, specific activity
Plasma	100	Lungs	36.5
Corpuscles	12.7	Spleen	30.8
Kidney	87.4	Stomach	25.9
Mucosa, small intestine	47.4	Heart	25.5
Liver	44.0	Brain	1.3

[a] Labeled phosphate given intravenously in an experiment lasting 215 minutes.

TABLE 83

Specific Activity of Inorganic Phosphate[a] Extracted from Rat Organs[77]

Organ	Duration of the experiment, hr.								Average specific activity during experiment[b]
	2	5	8.5	13	25	50	72	94	
Plasma	0.91	0.67	0.214	0.286	0.204	0.114	0.099	0.069	0.18
Liver	1.61	0.95	0.42	0.59	0.17	0.15	0.10	0.10	0.25
Kidney	1.10	0.78	0.43	0.57	0.18	0.16	0.096	0.10	0.24
Spleen	0.77	0.67	0.31	0.43	0.19	0.16	0.11	0.10	0.21
Mucosa, small intestine	0.72	0.51	0.35	0.51	0.15	0.13	0.11	0.11	0.19
Muscle	0.27	0.13	0.12	0.38	0.062	0.084	0.090	0.055	0.11
Testes	0.13	0.12	0.086	0.14	0.062	0.079	0.072	0.06	0.080
Brain	0.044	0.052	0.045	0.083	0.047	0.057	0.053	0.051	0.054

[a] Labeled phosphate given subcutaneously at start of experiment.
[b] Specific activity often denotes activity of 1 mg. phosphorus in arbitrary units (specific activity of plasma phosphorus being taken as 100). The values in the above table, however, denote percentage of administered ^{32}P present in 1 mg. phosphorus.

[76a] G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **15**, No. 7 (1940).

[77] G. Hevesy and J. Ottesen, *Acta Physiol. Scand.*, **5**, 237 (1943).

In 3.5 day old rats the specific activity of spleen inorganic phosphorus is almost identical with the corresponding figure for liver inorganic phosphorus; in full-grown animals, spleen is appreciably less permeable than liver to inorganic phosphorus. The permeability of red corpuscles to phosphate is discussed in Chapter XI.

TABLE 84

^{32}P in Inorganic Liver Phosphorus of the Rat[79]

Time, hours	Specific activity[a]	
	Full-grown rat	3.5 day old rat
0.5	2.3	2.12
1	2.8	2.39
2	2.1	2.14

[a] Per cent administered ^{32}P per milligram inorganic liver phosphorus.

When 300 mg. brain tissue was placed in 5 ml. Ringer solution, 7.5% of the ^{32}P added to the Ringer solution was recovered in the tissue after the lapse of 1 hour, the amount taken up increasing only slightly with time. Addition of glucose to the Ringer solution was found to increase the ^{32}P uptake to about twice the above value.[79a] As to the part played by cell division in the uptake of phosphate ions, preliminary experiments of Cohn and Brues[79b] indicate that the permeability rate is little affected by complete inhibition of mitosis.

The placental transfer of phosphatides was found to be a very slow process. Much more labeled phosphatide is synthesized by the fetus in 1 hour following injection into the maternal circulation of inorganic ^{32}P than can be transferred in 2 hours if tagged phosphatides are injected.[80] But phosphatide molecules pass capillary membranes or sinusoid walls and labeled phosphatides introduced into the circulation are subsequently recovered in the lymph from the thoracic duct. In one experiment over 20% of the radiophosphatides that left the blood stream were recovered from the lymph draining from the thoracic duct; in another case over 9% was recovered in 6 hours.[81]

[79] L. Ahlström, H. v. Euler, and G. Hevesy, *Svenska Vetenskapsakad. Arkiv Kemi,* **A19,** No. 9 (1944).

[79a] H. Schachner, B. A. Fries, and I. L. Chaikoff, *J. Biol. Chem.,* **146,** 95 (1942).

[79b] W. E. Cohn and A. M. Brues, *J. Gen. Physiol.,* **28,** 449 (1945).

[80] P. E. Nielson, *Am. J. Physiol.,* **135,** 670 (1941/42).

[81] W. O. Reinhardt, M. C. Fishler, and I. L. Chaikoff, *J. Biol. Chem.,* **152,** 79 (1944).

XXII. Effect of Muscular Exercise on Phosphate Interchange

In contradistinction to the effect of muscular exercise on the potassium interchange, muscular exercise has no appreciable effect on the interchange between the phosphate of muscle cells and that of plasma as seen in Table 85. The experiments were carried out by Hevesy and Rebbe[82] on rats weighing 170–210 g. The rats swam and rested alternately for 30 minutes. Thus the same technique was used as that applied in experiments with ^{42}K (see page 226). The total time of swimming and the time of the experiment are shown in Table 85.

TABLE 85

Distribution Coefficient of ^{32}P between Rat Gastrocnemius Muscle and Plasma of Equal Weight[82]

Time of swimming, hr.	Time of experiment, hr.	Distribution coefficient
0	7.1	5.52
0	7.6	4.90
0	7.7	5.54
0	7.1	6.80
2.3	7.1	5.25
3.4	7.5	5.27
3.2	6.8	4.38

Flock and Bollman found the ^{32}P content of inorganic phosphate in working muscle to be only 18% higher than in resting muscle.[83]

Prolonged contraction was also found by Sacks[83a] to be without appreciable effect on ^{32}P uptake by muscle. The effect of insulin on phosphate permeability is discussed in Chapter VIII.

The effect of stimulation on the amounts of phosphate, sodium, potassium and bromide taken up by the sciatic nerve of the cat was investigated with the aid of radioactive isotopes as indicators.[83b] Stimulation was effected by condenser shocks at a rate of 50 per second giving maximal motor reactions. In each case investigated, including the curarized animal, the stimulated nerve was found to take up more labeled ions than the resting nerve, the mean ratio of the uptakes being 1.55 ± 0.10.

[82] G. Hevesy and O. Rebbe, *unpublished data.*
[83] E. V. Flock and J. L. Bollman, *J. Biol. Chem.,* **152,** 371 (1944).
[83a] J. Sacks, *Am. J. Physiol.,* **142,** 621 (1944).
[83b] H. v. Euler, U. v. Euler, and G. Hevesy, *Acta Physiol. Scand.,* **12,** 261 (1946).

XXIII. Streamline Flow of Blood into Liver as Shown by Application of Radiophosphorus

When labeled phosphate was injected into the splenic vein, the concentration of tagged phosphorus in the left side of the liver was found by Hahn et al.[83c] to be several times greater than in the right side, within about 3 seconds of the injection. In contrast, when the same material is injected into a branch of the mesenteric vein, there is a markedly greater accumulation of labeled material on the right side of the liver. When the injection is made in the jugular vein and sufficient time (5 minutes) is allowed for complete mixing with the circulating blood, there are no demonstrable differences in concentration of tagged material in various transverse sections taken from the liver. The results indicate that there is a physiological bilaterality of blood flow in the portal vein which is streamline in nature. Splenic blood is thus largely sent to the left side and mesenteric blood to the right side of the liver.

XXIV. Interaction between Phosphate of Yeast Cells and Nutrient Solution

A. GENERAL REMARKS

Appreciable amounts of phosphate migrate from the nutrient solution into growing yeast cells. The interchange between phosphorus atoms of the yeast cells and the nutrient solution is, however, under aerobic conditions, very restricted.[84] This can best be shown when yeast is grown in a medium containing ^{32}P, the labeled yeast obtained being washed and shaken for a day with nonlabeled nutrient solution.[83d]

Some labeled phosphate is taken up in the course of the first 3 hours by resting yeast cells even in the absence of glucose in the outer solution (Malm[85]). This uptake is presumably not due mainly to interchange between cell phosphorus and solution phosphorus, but to accumulation of P during the metabolic processes shown to occur in the resting yeast cells in the early phase of the experiment. This conclusion is supported by the observation that under anaerobic conditions, in which endogenous fermentation is much less pronounced than respira-

[83c] P. F. Hahn, W. D. Donald, and R. C. Grier, Jr., Am. J. Physiol., **143**, 105 (1945).

[83d] G. Hevesy and K. Zerahn, Acta Radiol., **27**, 157 (1946).

[84] G. Hevesy, K. Linderstrøm-Lang, and N. Nielsen, Nature, **140**, 725 (1937).

[85] M. Malm, personal communication.

tion, markedly less ^{32}P is taken up in the absence of glucose than under aerobic conditions. The dependence of the uptake of ^{32}P by the cells upon the pH of the solution is found to be identical in both cases. Ahlström[86] found that nongrowing bakers' yeast takes up 2.5% of the ^{32}P added to the nutrient solution in the course of 48 hours, while growing yeast takes up as much as 50%. In these experiments 1 g. fresh yeast was suspended at 20° in 1 liter of water containing labeled potassium phosphate (pH equals 4.5 to 5) or in a nutrient solution of the same phosphate content and pH. Prior to the experiment the yeast was aerated in nonactive potassium phosphate solution for 18 hours to phosphorylate substrates possibly present in the cells.

B. AMOUNT OF LABELED PHOSPHORUS PENETRATING FROM CELLS INTO NUTRIENT SOLUTION

Yeast containing labeled phosphorus was repeatedly washed with inactive nutrient solution containing 1 g. K_2HPO_4 per liter; it was then shaken at 20° C. with inactive nutrient solution for 1 hour to 3 days.[83d] The activity of the nutrient solution was determined and compared with the activity of the cells. The yeast suspension averaged 8 mg. dry cell substance per milliliter. Some of the results obtained are shown in Table 86.

As shown in Tables 86 and 87 only about 1–4% of the total ^{32}P content of the cells migrates daily into the nutrient solution as inorganic ^{32}P. The actual loss of inorganic ^{32}P by living cells is presumably even lower than the above figure, since some autolysis of the cells takes place during the experiment and part of the ^{32}P present in the nutrient solution may originate from the autolyzed cells.

C. EFFECT OF RÖNTGEN RAYS ON PERMEABILITY OF YEAST

It has been repeatedly postulated that the effect of Röntgen rays on animal and plant tissues may be partly or wholly due to changed permeability of the cells. A change in permeability of tissue cells may influence the rate of entrance of substances into cells participating in metabolic and in synthetic processes that ultimately lead to cell division. Permeability changes may moreover affect the rate of elimination of waste products from cells.

With the aim of investigating the possible existence of such an effect, the permeability of yeast cells both to phosphate and to potas-

[86] L. Ahlström, *personal communication.*

sium was compared before and after irradiation, using ^{32}P and ^{42}K, respectively, as indicators.

Yeast cells are very resistant to the effect of Röntgen rays; this makes them well suited for such investigations. Large doses necessary to kill yeast cells can be expected to affect the cell permeability earlier than small doses. Yeast cells are several-hit systems, *i.e.*, they require several hits before a lethal effect is obtained. Such systems are killed more effectively by soft than by hard radiation, in view of the higher density of ionization produced by the former. Half the yeast cells were found to be killed by 19,000 r., when 60 kv. Röntgen rays were applied.

It is not advisable to carry out such permeability investigations by determining the amount of phosphate or potassium penetrating the yeast cells before and after irradiation. The growth of the yeast requires the uptake of substances essential to growing cells; obstruction of the growth by irradiation, however, diminishes the uptake of such substances. This diminution should not necessarily be interpreted as a consequence of altered permeability of the yeast cells; it can be and is presumably due to other factors than changes in the structure of the phase boundary. This argument no longer holds when the following procedure is used. Yeast is grown in the presence of labeled phosphate or labeled potassium; and correspondingly all or almost all phosphorus or potassium present in the yeast cells is labeled. The labeled yeast is then shaken with a nonradioactive nutrient solution, and the amount of ^{32}P or ^{42}K migrating from the cells into the nutrient solution is determined. Simultaneously, experiments are performed in which yeast cells are irradiated previous to shaking them with inactive nutrient solution. If no difference is found in the amount of ^{32}P and ^{42}K, respectively, migrating from the cells, we must conclude that the irradiation had no effect on the permeability of the yeast.

To test the effect of Röntgen rays on phosphorus interchange, 1 ml. yeast (*Saccharomyces cerevisiae*) suspension under aerobic conditions was placed in a glass tube of 8 mm. diameter, and the tube was irradiated with a nonfiltered 60-kv. radiation emitted by a copper anticathode for 30 minutes. The total dose administered was about 30,000 r. Comparison of the percentage of surviving cells in the irradiated samples with the percentage in control preparations showed that under the action of the radiation all but 10% of the cells were killed. Both the irradiated and the nonirradiated cells were washed 4 times with inactive

nutrient solution; subsequently, the washed cells were shaken for 1 to 2 days with inactive nutrient solution at *ca.* 15° C. The suspension contained 8–10 mg. of dry yeast per ml. The results obtained are shown in Table 86.

<div align="center">TABLE 86</div>

<div align="center">Inorganic ^{32}P Migrating from Cells Irradiated with Röntgen Rays
and from Nonirradiated Cells into Nutrient Solution[83d]</div>

Sample	Time of experiment, days	Inorganic ^{32}P migrated, %
Control..........................	1	1.8
Irradiated.....................	1	1.2
Control......................	2	2.7
Irradiated.....................	2	1.7
Control......................	1	1.3
Irradiated.....................	1	0.9
Control......................	2	1.7
Irradiated.....................	2	1.1
Control......................	1	1.7
Irradiated.....................	1	0.8
Control......................	2	2.1
Irradiated.....................	2	1.2

As seen in the table, a substantial x-ray dose that kills most of the yeast cells influences the permeability to a minor extent only, the amount of inorganic ^{32}P migrating through the phase boundary between cell and nutrient solution in irradiated yeast being somewhat less (two-thirds) than in the controls.

<div align="center">D. EFFECT OF ULTRAVIOLET RADIATION ON EXTRUSION
OF ^{32}P BY YEAST</div>

Very divergent results were obtained after irradiating yeast cells with ultraviolet. In these experiments, a mercury discharge lamp (type Osram Hg Q 500) was used to irradiate a quartz vessel containing 25 cc. yeast suspension. The distance of the vessel from the lamp was 25 cm. Table 87 illustrates that appreciable amounts of inorganic and organic acid-soluble labeled phosphorus accumulate in the suspension solution.

Irradiation was found to increase strongly the amount of organic and inorganic acid-soluble ^{32}P moving from the yeast into the nutrient solution, much of the acid-soluble ^{32}P being possibly formed by the action of ultraviolet radiation on the ribosenucleic acid content of the yeast.

TABLE 87

Inorganic and Acid-Soluble ^{32}P Extruded from Yeast Cells
into Nutrient Solution in Twenty-Four Hours[83d]

| | Per cent extruded | |
Treatment[a]	Inorganic ^{32}P	Acid-soluble ^{32}P
Nonirradiated..................	1.75	4.10
Irradiated 5 min..............	2.54	5.60
10	3.88	8.44
15	7.74	16.8
30	11.7	24.5

[a] Percentage survival after 5 minutes irradiation = 59, after 10 minutes irradiation = 8, after 15 minutes irradiation = 0.

The wide difference in behavior shown by the cells irradiated with Röntgen rays and those irradiated with ultraviolet light may be attributed to two different causes. Much greater energy is necessary to obtain a lethal effect when irradiating yeast with ultraviolet radiation than when irradiating with Röntgen rays. Applying a Röntgen radiation of 1.5 Å, 0.8×10^{-6} ergs have to be dissipated per cubic micron to obtain a reduction of 50% in living yeast cells. Applying ultraviolet light of 2537 Å, 175×10^{-6} ergs are needed to produce the same effect. Beside the much greater energy used in the experiments with ultraviolet rays, the fact that ultraviolet radiation is absorbed selectively by some of the cell constituents may be of significance in producing enhanced migration of ^{32}P from the yeast cells.

In one experiment, phosphate of the nutrient solution was replaced by 50 millimoles neutralized sodium arsenate per liter by shaking the active yeast cells with an inactive nutrient solution. This replacement resulted in a decrease to half its normal value of the migrating inorganic ^{32}P.

XXV. Permeability of Capillaries to Colloids

A study of the permeability of capillaries to colloids has been made by Cope and Moore[87] (see also Moore, Tobin and Aub[88]), who injected

[87] O. Cope and F. D. Moore, *J. Clin. Investigation*, **23**, 241 (1944).
[88] F. D. Moore, L. H. Tobin, and J. C. Aub, *J. Clin. Invest.*, **22**, 161 (1943).

radioactive colloids into the blood stream of dogs and measured their appearance in the lymph from various areas. The radioactive colloidal dyes used were brominated derivatives of trypan blue and Evans blue (T-1824). The radioactivity is due to the presence in the molecule of two atoms of radiobromine (^{82}Br). The rise in concentration of radio-activity in the lymph following injection of the radioactive solution is slow and equilibrium is not reached in the course of 7 hours, half of the tagged dye disappearing from the plasma in 15 hours. When these figures are compared with those obtained for the bromide ions, it is found that the presence of bromide ions can be detected in the leg and in the cervical and thoracic lymph within 5 minutes after intravenous injection and that equilibrium has been reached between lymph and blood serum in 20 minutes.

In a pathologic state, the capillary membrane may become as permeable to colloids as it formerly was only to ions. It is found that, following a scalding of a leg, the concentration of radioactive colloids in the lymph from this leg rises abruptly and approaches that encountered after injection of the inorganic ion. After a burn, the specific activity of lymph may be found to be higher than the corresponding value of blood serum. This result indicates a preferential escape through the capillary wall of one fraction of plasma protein (see also Chapter VIII).

When radioactive dibromotrypan blue and radioactive dibromo Evans blue were injected into tumor-bearing mice, some of the dye was found in many tissues and organs. The uptake of dye by tumors did not appear to be selective. In experiments taking 3 to 30 hours 1 g. tumor tissue took up about 1/7 of the amount taken up by the liver tissue and only slightly more than 1 g. muscle tissue.

Data on the permeability of red corpuscles to various substances are found in Chapter XI.

Turnover Studies

I. Specific Activity and Rate of Renewal

A. CONCEPT OF SPECIFIC ACTIVITY

In the work with radioactive tracers the percentage of the administered dose found in one gram of tissue is often stated; this value magnitude indicates the distribution of the tracer in the different organs. Often data are also given on the percentage of the tracer, for example, of the ^{32}P administered present as phosphatide phosphorus per gram tissue. It is often important to know the percentage of the ^{32}P administered present per milligram ·phosphatide phosphorus. This value is denoted as *specific activity* of the phosphatide phosphorus and states the value determined at the end of the experiment. If we want to know the specific activity prevailing on the average during the experiment we have to carry out experiments of different duration, plot the values obtained as ordinates against the time as abscissa, and evaluate the average.

In some cases it may not be convenient to express the specific activity as percentage of the activity administered. In cases such as these, it might prove advisable to employ relative specific activity values which are the ratio of two specific activities; for example, we express the specific activity of muscle creatine phosphorus with respect to the specific activity of the plasma inorganic phosphorus, assuming the specific activity of the plasma inorganic phosphorus to be 100. Alternatively the specific activity of the tissue inorganic phosphorus can be assumed to be equal to 100.

B. CONCEPT OF RATE OF RENEWAL

The application of isotopic tracers reveals the constant renewal of most of the body constituents. Degradation and formation are mostly interpreted as independent processes. Some of the apatite-like crystals of the skeleton, or parts of them, are dissolved in the plasma or lymph; new crystals or molecular layers building up the crystals are formed.

These are independent processes. Under physiological conditions the mineral constituents of the skeleton of the full-grown organism remain about constant during a restricted interval; the rate of degradation thus corresponds closely to the rate of formation and the determination of one of these values shows the other rate as well.

To consider another example, the thyroid gland supplies thyroxine to the circulation from which it is carried into the organs and metabolized. The rate of renewal, also denoted as rate of rejuvenation or rate of turnover, of the plasma thyroxine is determined (see page 365) by measuring the rate of disappearance of labeled protein-bound iodine injected into the circulation. The amount of thyroxine removed from the plasma, where a concentration of this substance remains during a restricted interval constant, corresponds to the amount of thyroxine carried from the gland into the plasma and thus by measuring the rate of disappearance of thyroxine from the plasma we arrive at the amount of thyroxine given off by the gland to the circulation. The rate of replacement of thyroxine molecules present in the plasma does not, however, permit calculation of the amount of thyroxine built up during a time interval in the thyroid since, in addition to what is secreted, an appreciable renewal of thyroxine may be taking place in the gland.

To consider another example, during muscular exercise, creatinephosphoric acid phosphate molecules split off phosphate, while under resting conditions a formation of creatinephosphoric acid molecules takes place. The application of ^{32}P as an indicator shows that, even in the resting muscle, a rapid formation of new creatinephosphoric acid molecules takes place. Let us assume for the sake of simplicity the intracellular inorganic phosphorus content of the muscle cells to be constant during the experiment and the creatinephosphoric acid phosphorus to have a specific activity of 10% of the specific activity of the cellular inorganic phosphorus at the end of the experiment. This result indicates that 10% of the creatinephosphoric acid molecules are formed in the course of the experiment.

We can determine the rate of renewal instead of measuring the rate of formation of labeled molecules by measuring the rate of their disappearance. Let us, for example, consider labeled nucleic acid. In the later part of a protracted experiment, the specific activity of the nucleic acid phosphorus will be higher than that of the tissue inorganic phosphorus. As renewal of the nucleic acid molecules takes place incessantly in the animal tissue, a successive release of the nucleic acid ^{32}P by the

tissues will take place; during the latter part of the experiment, the gradient of ^{32}P is directed from the tissues into the plasma. If the experiment is protracted, the nucleic acid is bound to lose much of its ^{32}P content, the amount lost being a function of the rate of renewal of the nucleic acid. Clearly molecules which are getting labeled at a slow rate only lose their label slowly and vice versa.

The rate of renewal is advantageously determined from the rate of disappearance when we inject labeled thyroxine or other labeled molecules into the circulation as described above. Usually, however, the rate of renewal is determined by measuring the rate of formation of the labeled molecules.

The determination of the rate of renewal is one of the most important applications of isotopic tracers. When attempting this, many obstacles are met. The rate of penetration of the tracer into the cells in which the compound is turned over may take place at a rate which is slow compared with the rate of formation of the labeled compound. The organ in which the labeled molecules are located may not be identical with the place of their formation. Furthermore, unknown precursors may become labeled at a comparatively slow rate — thus the early phases of the turnover are not indicated by the tracer.

Plasma phosphatides, as shown by Chaikoff *et al.*, originate exclusively from the liver. By comparing the specific activities of the plasma phosphatide phosphorus and plasma inorganic phosphorus, we will thus not arrive at turnover figures for the plasma phosphatides. These can be obtained, however, by measuring the rate of disappearance of labeled plasma phosphatides introduced into the circulation. The notion of the turnover rate of plasma phosphatides indicates the replacement rate of plasma phosphatide molecules by liver phosphatide molecules and is thus unambiguous; not so the notion of the turnover rate of liver phosphatides. It is possible that the phosphate group of the lecithin molecule is renewed at a rate different from that of the choline molecule or the fatty acid. The different constituents of chlorine or fatty acids may again be renewed at a different rate. Therefore, when discussing the rate of renewal of the liver phosphatides, we must state the moiety of the molecule to be considered.

Let us assume that glycerophosphate phosphorus is the precursor of lecithine phosphorus. If ^{32}P is incorporated into glycerophosphate at a much more rapid rate than into lecithin, the comparison of the specific activity of lecithin phosphorus and tissue inorganic phosphorus

will lead to correct values as to rate or renewal of the lecithin phosphorus. When the above assumption does not hold, lecithin molecules will, in the early phases of the experiment, renew their phosphorus atoms without the tracer's indicating the process. Just as a slow rate of migration of the indicator to the place of formation of the labeled product will be responsible for underestimation of turnover rate, the existence of a precursor of the above-mentioned type will also lead to low turnover figures. As long as the mechanism of phosphatide formation is not known, it is advisable in turnover studies to determine the percentage ratio of the specific activities of the phosphatide phosphorus and the intracellular inorganic phosphorus or that of the phosphatide phosphorus and terminal adenosine triphosphate phosphorus (see page 257) of the tissue. This ratio indicates the percentage of incorporation of cellular inorganic phosphorus into the phosphatide molecule during the time of the experiment. After elucidation of the problem of phosphatide precursors, it will be possible to apply the possibly necessary corrections to arrive at the value of the percentage rate of renewal of the phosphatide phosphorus from the percentage incorporation rate of the cellular inorganic phosphorus.

In contrast to the first-mentioned case (permeability) in which corrections can be made by determining the specific activity of the intracellular inorganic phosphorus, for example, in the last-mentioned case (precursor), the nature and rate of formation of the precursor must be known to enable us to arrive at correct turnover figures.

It is difficult to interpret the results obtained in turnover experiments when many or all the molecules of the compound considered have been renewed, because many or all the molecules may have turned over several times. Therefore, it is advisable not to renew more than about one-fifth of the molecules and to choose the length of the experiment accordingly. This will much facilitate the calculation of turnover rate.

C. CALCULATION OF TURNOVER OF SUBSTANCE INTRODUCED INTO THE CIRCULATION

An exact determination of turnover rate can easily be carried out in cases in which part of the blood plasma of the animal is replaced by plasma containing labeled molecules of certain compounds such as, for example, phosphatides or protein-bound iodine compounds. In such cases it can be assumed that the labeled molecules leave the plasma at random, the decrease of the labeled phosphatide taking place according

to the equation:

$$L_t = L_0 \, e^{-st}$$

where s is the constant of disappearance (analogous to the radioactive decay constant). It is also assumed that the removed labeled phosphatides are replaced by phosphatides carried from the tissues into the plasma and that the phosphatide concentration of the plasma remains constant during the experiment. We are interested in the turnover rate, *i.e.*, in the amount of phosphatides removed from the plasma during a given time and replaced by tissue phosphatides. When calculating this amount from radioactive data we must take into consideration that due to successive disappearance of labeled phosphatide the sensitivity of the indicator constantly increases. In the following, the calculation of turnover rate carried out by Zilversmit *et al.*[1] is given with the application of this calculation to the determination of the amount of phosphatides turned over in the plasma per unit time. The turnover time is taken as the time required for the appearance or disappearance of the phosphatides, for example, present in the plasma. If the rate of disappearance of phosphatides from the plasma is p, the amount of phosphatides present in the plasma is r, and the amount of radioactive phosphatides (measured by their activity) is x, then:

$$\frac{dx}{dt} = -p\frac{x}{r}$$

and on integration:

$$\ln \frac{x}{r} = \ln c - \frac{p}{r} t,$$

It is clear that a plot of $\ln x/r$ against t will yield a straight line whose slope will be $-p/r = 1/t_t$, where t_t denotes the turnover time. The values in Table 88 were found by Zilversmit *et al.* for the turnover time and turnover rate of phosphatides in the plasma of the dog.[1] By a very similar consideration Hevesy and Hahn[2] determined the turnover time of phosphatides present in the plasma of the rabbit to be about 5 hours.

[1] D. B. Zilversmit, C. Entenman, and M. C. Fishler, *J. Gen. Physiol.*, **26**, 325 (1943). H. Branson, *Bull. Math. Biophys.*, **8**, 159 (1946); *ibid.*, **9**, 93 (1947).

[2] G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **15**, 6 (1940).

TABLE 88

Turnover Time and Rate of Phosphatides in Plasma of the Dog

Plasma volume, ml.	535	323	339
Per cent of body wt.	6.6	4.9	6.0
Turnover time, hr.	9.3	7.2	7.8
Turnover rate, mg. phosphatide P per hr.	7.0	5.2	8.0

D. GENERAL CASE OF CALCULATION OF TURNOVER RATE

Zilversmit et al.[1] also calculated the rate of turnover for the general case of the formation of a substance B containing the labeled atom from its immediate precursor A. Using the formulation given above, the amount of radioactivity that would be converted into P per unit time is $pf(t)$ and the amount of radioactivity lost from B per unit time is $p(x/r)$. Therefore, the rate of change in amount of radioactivity in B in a tissue per unit time is:

$$\frac{dx}{dt} = pf(t) - p\frac{x}{r} = p\left[f(t) - \frac{x}{r}\right]$$

or:

$$r\frac{d(x/r)}{dt} = p\left[f(t) - \frac{x}{r}\right]$$

since r is constant, and:

$$\frac{\dfrac{d(x/r)}{dt}}{f(t) - \dfrac{x}{r}} = \frac{p}{r} = \text{a constant,}$$

where $d(x/r)/dt$ measures the slope of the specific activity–time curve of B.

The following relation was deduced between the specific activity (s.a.) (see Fig. 48) of compound B and the specific activity of its precursor A. At any time the slope of the "s.a. – time" curve of B is proportional to the difference between the specific activity of A, i.e., $f(t)$, and the specific activity of B, i.e., x/r. The application of this relation in the case in which a single dose of labeling agent is administered is illustrated in Figure 48.

The following criteria for an immediate precursor of the compound to be turned over are emphasized. (1) If the slope of the "s.a. – time" curve of B is positive (see Fig. 48), i.e., before the specific activity of B reaches its maximum, $f(t) - x/r$ must be positive. This means that the specific activity of the immediate precursor A is greater than that of the compound B before the latter reaches its maximum specific activity. (2) After B has reached its maximum specific activity, the slope of the "s.a.–time" curve of B is negative, and therefore the specific activity of the compound is greater than that of its precursor. (3) At the time when B has reached its maximum specific activity the slope of the "s.a.–time" curve of B is zero and therefore the specific activity of the immediate precursor A equals that of compound B at that time.
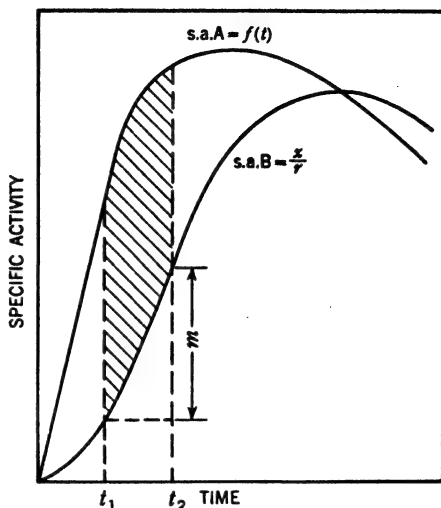
Fig. 48. Illustration of specific activity (s.a.) – time relations of precursor A and product B.[1]

E. CALCULATION OF INTERMEDIATE CARBON DIOXIDE PRODUCTION ON BASIS OF DILUTION OF ADDED LABELED CARBON DIOXIDE

As an example of the calculation of an intermediate product (formed or utilized) on the basis of dilution of the added labeled compound, the calculation of intermediate carbon dioxide production during the conversion of glucose into acetic acid, as given by Barker and Kamen,[3] will be discussed.

To calculate the intermediate carbon dioxide production we shall assume that the only pertinent reactions are: (a) glucose (^{12}C) $\rightarrow$ CO$_2$(^{12}C) and (b) CO$_2$(^{12}C + ^{14}C) $\rightarrow$ acetic acid (^{12}C + ^{14}C).

The carbon dioxide will always consist of a mixture of ^{14}CO$_2$ and ^{12}CO$_2$. Let x be the quantity of ^{14}CO$_2$ per unit volume at any time during the fermentation and x_0 the initial, and x_f the final, ^{14}CO$_2$.

[3] H. A. Barker and M. D. Kamen, *Proc. Natl. Acad. Sci. U. S.*, **31**, 219 (1945).

Further, let V represent the amount of $^{12}CO_2$ plus $^{14}CO_2$ converted to acetic acid at any time; V also equals the amount of $^{12}CO_2$ formed from glucose since there is no net change in carbon dioxide. V_a is the constant amount of carbon dioxide present throughout the fermentation and V_f is the total carbon dioxide formed or utilized during the experiment.

When a small quantity (ΔV) of $^{14}CO_2 + {}^{12}CO_2$ is converted to acetic acid and an equal amount of $^{12}CO_2$ is formed from glucose, the decrease in $^{14}CO_2$ ($- \Delta x$) is given by the expression:

$$- \Delta x = \frac{\Delta V}{V_a + \Delta V} \times x$$

This means that the $^{14}CO_2$ (x) is decreased by a fraction equal to the carbon dioxide used (ΔV) divided by the total carbon dioxide present ($V_a + \Delta V$). Dividing by x we obtain:

$$\frac{- \Delta x}{x} = \frac{\Delta V}{V_a + \Delta V}$$

In the limit as ΔV is decreased:

$$\frac{- dx}{x} = \frac{dV}{V_a + dV} = \frac{dV}{V_a}$$

Integrating this expression between the limits x_0 and x_f for x, and 0 and V_f for V, we find:

$$- \int_{x_0}^{x_f} \frac{dx}{x_1} = \ln \frac{x_0}{x_f} = \frac{1}{V_a} \int_0^{V_f} dV = \frac{V_f}{V_a}$$

or:

$$V_f = 2.3 \, V_a \log \frac{x_0}{x_f}$$

V_f has the same units as V_a. V_f must be divided by the quantity of glucose fermented (moles acetic acid formed divided by 2.65) to give carbon dioxide production per unit glucose. When carrying out this calculation it is assumed that instantaneous mixing between intracellular and extracellular carbon dioxide takes place. Should that not be the case, the calculation leads to too low figures for the carbon dioxide formation.

II. Turnover of Phosphorus-Containing Carbohydrates

A. RATE OF RENEWAL OF LABILE PHOSPHORUS COMPOUNDS OF MUSCLE TISSUE

In the determination of the rate of renewal of creatinephosphoric acid, *i.e.*, the rate of rephosphorylation of creatine molecules in muscle tissue we compare the radioactivity of 1 mg. of creatinephosphoric phosphorus at the end of the experiment with the average activity of 1 mg. of inorganic intracellular phosphorus prevailing during the experiment. If this ratio is found to be 0.2, we can conclude that 20% of the creatinephosphoric acid molecules present were renewed in the course of the experiment. It is not the activity of inorganic *intracellular* phosphorus that we determine experimentally, but the activity of inorganic *tissue* phosphorus. We calculate the former quantity from the latter by making the following assumptions:[3a]

(*1*) Extracellular space (chloride space) of muscle has the same free phosphate concentration as plasma, and (*2*) rate of interchange between plasma phosphate and extracellular phosphate is a comparatively rapid process, that is, the specific activity of extracellular inorganic phosphorus is identical with that of free (inorganic) plasma P. If the specific activity of the plasma inorganic phosphorus is equal to 1000 and that of the tissue inorganic phosphorus to 100 counter units, and if 20 per cent of the muscle tissue is composed of extracellular fluid, the inorganic phosphorus content of the plasma is 3 milligrams per cent and that of the muscle tissue is 15 milligrams per cent; then 0.6 milligrams of 15 milligrams inorganic phosphorus will be extracellular phosphorus; the extracellular phosphorus will have an activity of 600, and the tissue inorganic phosphorus of 1500, counter units. The 14.4 milligrams inorganic cellular phosphorus will thus have an activity of 900 units; or the specific activity of the inorganic cellular phosphorus (62.6) will amount to only 63 per cent of the experimentally determined specific activity of tissue inorganic phosphorus.[3b] In experiments of shorter duration, which are often of great interest, the difference between the specific activity of the inorganic tissue phosphorus and the inorganic cellular phosphorus may become very appreciable. In such experiments even the assumption of equal specific activities for the inorganic extracellular phosphorus and the inorganic plasma phosphorus will not be justified.

One way of studying rate of renewal of phosphate compounds in muscle is by removal of the extracellular phosphate by perfusing muscle with saline. Kalckar and his associates[4] compared, under these circum-

[3a] G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **15**, 5 (1940).

[3b] G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **15**, 7 (1940).

[4] H. M. Kalckar, *J. Biol. Chem.*, **154**, 267 (1944). H. M. Kalckar, J. Dehlinger, and A. Mehler, *ibid.*, **154**, 275 (1944).

stances, the specific activity of phosphocreatine phosphorus and of adenyl pyrophosphate phosphorus with that of the inorganic phosphorus, which is exclusively of intracellular origin.

That in perfused muscle the specific activity of inorganic phosphorus is much lower than in unperfused muscle is seen from Table 89. We must expect that perfusion will not influence the specific activity of the phosphocreatine and 2,3-pyrophosphate phosphorus, since these compounds are located in the cells.

Only 20 minutes after an intravenous injection of labeled phosphate, the creatine phosphorus, both of the rabbit's and the frog's (at 20°C.)

TABLE 89

Specific Activity of the Phosphorus Fractions Isolated from the Skeletal Muscle of the Rabbit[4]

Type of injection of [32]P	Time after injection, min.	Inorganic P		Phosphocreatine P		2,3-Pyrophosphate P	
		Un- perfused	Perfused	Un- perfused	Perfused	Un- perfused	Perfused
Intravenous........	30	235	100	61	61	58	57
Intraperitoneal	180	160	100		100	94	94

skeletal muscle, was found to have a specific activity corresponding to 60% of that of the inorganic phosphorus. The same results were obtained for the labile phosphorus of adenyl pyrophosphate, conforming with those of early experiments,[2,5-5b] and indicating that the rate of renewal of the labile phosphorus compounds is a rapid process, in contrast to the rate of penetration of labeled phosphate into the muscle cells.

In liver and red corpuscles (see page 448), the rate of rejuvenation of the labile adenyl pyrophosphate phosphorus is of the same order of magnitude as that present in resting muscles. Five minutes after intravenous injection of labeled phosphate, the specific activity of the pyrophosphate phosphorus amounts to 83% of that for the inorganic phosphorus,[4] and a similar figure was found for pyrophosphate present in red corpuscles of the rabbit in experiments taking 30 minutes.[3b,6]

[5] G. Hevesy, L. Hahn, and O. Rebbe, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **16**, No. 8 (1941).

[5a] G. Hevesy and O. H. Rebbe, *Nature*, **141**, 1097 (1938).

[5b] G. Hevesy, *Les Pris Nobel 1940–1944*, Stockholm, 1946.

[6] G. Hevesy and A. H. W. Aten, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **14**, No. 5 (1939).

Flock and Bollman,[7,8] using myosin adenosinetriphosphatase (ATPase) as a tool for differentiating between the two labile groups of ATP, have found, in experiments lasting one hour, a higher ^{32}P concentration in the terminal phosphate group than in the second phosphate group (Table 90). After the lapse of one day, the specific activity of the

TABLE 90

Specific Activity of the Three P Atoms of Adenosine Triphosphate[8]

Fraction	Specific activity
Total inorganic muscle P................	100
Terminal adenosine triphosphate P........	29
Second adenosine triphosphate P..........	21.6
Third adenosine triphosphate P...........	0.9
Creatine P..............................	24

two labile phosphorus atoms of ATP (see also Sacks and Altschuler[9]) and that of the creatine phosphorus was found to be but slightly lower than the activity of the inorganic phosphorus. The low rate of renewal of the third ATP phosphorus atom had been previously observed by Korzybski and Parnas.[10]

In the turnover of the labile phosphate of ATP which occurs in the working muscle, the uptake of ^{32}P is increased only slightly above that of the resting muscle, even after 180 contractions per minute for one hour. The turnover rate of the labile phosphate compounds is already so high in resting muscles that it is difficult to discover any substantial increase in the turnover during or after muscular contraction. The rate of rejuvenation of adenyl pyrophosphate phosphorus and of phosphocreatine amounts to $20-30$ μg. per minute per gram muscle.[4] The rate of penetration of ^{32}P into the cells of working muscle is not substantially different from the corresponding rate in resting muscle (see page 240).

The rate of renewal of creatine phosphate and ATP molecules in cardiac muscle slices was investigated by Furchgott and Shorr.[7,11] A

[7] R. F. Furchgott and E. Shorr, *Federation Proc.*, **2**, 13 (1943).

[8] E. V. Flock and J. L. Bollman, *J. Biol. Chem.*, **152**, 371 (1944).

[9] J. Sacks and E. H. Altschuler, *Am. J. Physiol.*, **137**, 750 (1942).

[10] T. Korzybski and J. K. Parnas, *Bull. soc. chim. biol.*, **21**, 713 (1939).

[11] R. F. Furchgott and E. Shorr, *J. Biol. Chem.*, **151**, 65 (1943).

quantitative renewal of creatine phosphoric and terminal ATP phosphorus was found to take place within 30 minutes at 37.5°C.

The extent of renewal of creatine and of the labile phosphorus of ATP molecules was found, even at 2°, to be appreciable, amounting to about one-third the values found at 37.5°. The rate of renewal in these experiments is calculated from the ratio of the specific activities of creatine phosphorus or of labile ATP phosphorus, and that of inorganic phosphorus after a prolonged washing of the tissue slices to remove the inorganic extracellular phosphorus. Such a procedure may lead to decomposition of the less active phosphorus compounds with formation of inorganic phosphorus and thus to a decrease in the specific activity of the inorganic phosphorus present after washing is terminated. Should this be the case, it will result in too high values for the renewal figures.

As for the labile phosphate split off ATP, it was found that it contained a mixture of the terminal, highly active, and a second, less active fraction. On splitting off the terminal phosphate with an enzymic crayfish muscle suspension free of phosphate, Furchgott and Shorr obtained, after 30 minutes, a fraction having a specific activity of 100, while only one-half of the remaining labile phosphorus of ATP underwent renewal in the course of the experiment.

In the boundary between the extracellular and the intracellular phases, a more or less continuous drop in the concentration of the introduced labeled phosphate may take place. If a phosphorus compound is built up inside the phase boundary, it is very difficult to determine the specific activity of the inorganic phosphorus that has been utilized in the synthetic process resulting in the compound. Sacks,[12,13] found that, in experiments on fasted animals taking a few hours or less, the specific activity of the glucose-6-phosphate phosphorus was very much higher than those of the phosphocreatine, ATP, and fructose-6-phosphate phosphorus, as seen in Tables 91 and 92. See also the results obtained by Kalckar and associates.[4] This result suggests that in the synthesis of the labeled glucose-6-phosphate molecules no intracellular free phosphate, but phosphorus of much higher activity, is utilized, and thus that the synthesis of this compound takes place outside the muscle cells, i.e., inside the extracellular space or possibly inside the phase boundary. The high ^{32}P content of glucose-6-phosphate is never found in fed animals (in the postabsorptive phase) but only in fasted animals. The

[12] J. Sacks, *Am. J. Physiol.*, **142**, 145 (1944).
[13] J. Sacks, *Am. J. Physiol.*, **143**, 157 (1945). See also reference (4).

incorporation of non-intracellular ^{32}P in glucose-6-phosphate phosphorus is thus possibly involved in the process of entry of glucose into cells of the fasting animal.

The rate of renewal of hexose monophosphate was found to be considerably lower than that of pyrophosphate or creatine phosphate.[5,14]

TABLE 91

Time Course of Uptake[a] of ^{32}P by Acid-Labile Groups of Adenosine Triphosphate, Fructose-6-phosphate, and Glucose-6-phosphate in Resting Muscles of Fasted Cats[12]

2 hr. after ^{32}P			4 hr. after ^{32}P			24 hr. after ^{32}P		
ATP	Fructose-6-phosphate	Glucose-6-phosphate	ATP	Fructose-6-phosphate	Glucose-6-phosphate	ATP	Fructose-6-phosphate	Glucose-6-phosphate
38	65	124	75	31	114	193	132	84
80	84	202	84	14	167	167	122	102
66	27	149	52	19	170	165	121	88
79	37	189	77	25	219	224	150	121
88	102	209	78	60	228			
			65	56	250			
			70	45	217			
70	*63*	*175*	*72*	*36*	*195*	*187*	*131*	*99*

[a] Values are expressed in terms of counts per minute per milligram P, calculated to the basis of 10⁶ counts injected per minute per kilogram body weight, as of the day of measurement. Average values are italicized.

TABLE 92

Uptake[a] of ^{32}P by Fasting Muscles of Frogs[12]

ATP	Fructose-6-phosphate	Glucose-6-phosphate	ATP	Fructose-6-phosphate	Glucose-6-phosphate
2 hr. after ^{32}P			24 hr. after ^{32}P		
73	82	348	233	142	134
23	62	152	306	155	141
84	154	435	152	100	99
136	144	472	106	62	65
79	*111*	*352*	*199*	*115*	*109*

[a] Values are expressed in terms of counts per minute per milligram P, calculated to the basis of 10⁶ counts injected per minute per kilogram body weight, as of the day of measurement. Average values are italicized.

[14] J. Sacks, *Am. J. Physiol.*, **129**, 227 (1940).

Analysis of frog muscle at low temperature shows that the hexose monophosphate in skeletal muscle attained only a very low concentration of ^{32}P. It appears as if some enzymes converting glycogen and

TABLE 93

Effect of Stimulation, Recovery and Glucose Administration on ^{32}P Turnover[a] in Muscles of Cats in Postabsorptive State[15]

Phospho-creatine	ATP	Fructose-6-phosphate	Glucose-6-phosphate	Phospho-creatine	ATP	Fructose-6-phosphate	Glucose-6-phosphate
Resting				Stimulated			
119	98	61	77	99	102	41	54
130	132	76	59	138	125	69	77
170	143	...	47	204	160	105	44
157	150	133	73	104	115	...	76
144	*131*	*90*	*64*	*136*	*126*	*72*	*63*
Resting				Stimulation and recovery			
146	145	73	58	308	282	100	72
137	140	64	45	207	177	126	73
208	172	110	67	263	234	168	83
124	115	75	55	157	174	128	65
154	*143*	*81*	*56*	*234*	*217*	*131*	*73*
After glucose							
67	58	55	38	89	85	75	74
131	76	51	37	147	118	41	59
89	87	67	70	90	101	71	75
80	87	67	60	124	108	78	70
46	51	32	47				
39	56	17	30				
105	99	..	38				
75	92	50	94				
79	*76*	*48*	*53*	*113*	*103*	*66*	*70*

[a] Values are expressed as counts per minute per milligram P, calculated to the basis of one million counts per minute injected per kilogram body weight. Average values are italicized.

[15] J. Sacks, *Am. J. Physiol.*, **142**, 621 (1944).

phosphate to hexose monophosphate were inactive in frog muscle at low temperature.[4]

Sacks [15,16] also studied the effects of prolonged stimulation, recovery, and glucose administration on the uptake of ^{32}P by the phosphorus compounds of muscle of cats in fasting and postabsorptive state. Prolonged contraction was found to be without effect on either the uptake of ^{32}P by any of the acid-soluble organic phosphorus compounds of muscle (see Table 93), or its distribution among them. In recovery from prolonged activity all the organic phosphorus compounds investigated were found to show a higher ^{32}P content, as seen in Table 93.

Administration of glucose reduces the turnover rate in postabsorptive state of phosphocreatine, adenosine triphosphate, and fructose-6-phosphate, but not that of glucose-6-phosphate. In fasting state, the administration of glucose does not affect the turnover rate of phosphocreatine and adenosine triphosphate.

B. EFFECT OF INSULIN ON PHOSPHORUS TURNOVER IN MUSCLE

In the experiments of Sacks,[13] five insulin units were employed per kilogram cat weight. Half an hour after administration of labeled sodium phosphate to a cat by subcutaneous injection, 1.5 g. glucose was given per kilogram; after a lapse of a further half-hour, insulin was administered. The cat was killed 105 minutes after injection of the tracer phosphate. The results of the experiment, shown in Table 94, indicate that both in resting and stimulated muscles insulin brings about an increase in the ^{32}P content of phosphocreatine, adenosine triphosphate, and fructose-6-phosphate fractions. In the postabsorptive state, but not in the fasting state, insulin increases the ^{32}P content of glucose-6-phosphate as well. Sacks suggests that in the latter case we are presumably concerned (see page 258) with an influx of glucose, in which nonintracellular ^{32}P participates, and in the postabsorptive case with a renewal of the glucose-6-phosphate molecules, intracellular phosphorus participating.

A rather small amount of phosphorylation is observed in relation to the total probable glucose absorption. Two possible interpretations are given by Sacks: (1) the absorption of glucose by the muscle fiber does not involve the entry of a phosphate group into the cells, or (2) glucose-6-phosphate is not involved in the principal mechanism of glucose absorption by the resting muscle.[16] The first interpretation is in accord-

[16] J. Sacks, *Science*, **98**, 388 (1943).

ance with the results arrived at by Kjerulf-Jensen and Lundsgaard[17] in their study on phosphate exchange between plasma and muscle tissue with artificially perfused hind limb preparations in which labeled phosphate was employed. They found that both before and after addition of insulin the quantity of phosphate exchanging per unit time

TABLE 94

Effect of Insulin on P Turnover[a] in Muscles of Cats Fed Glucose[13]

State of animals	Phospho-creatine	ATP	Fructose-6-phosphate	Glucose-6-phosphate
Fasting, resting.....................	103	77	66	514
Fasting, resting, given insulin.........	316	189	138	482
Fasting, stimulated and recovering.....	119	100	55	606
Fasting, stimulated and recovering, given insulin...........................	271	205	203	733
Postabsorptive, resting..............	75	76	49	52
Postabsorptive, resting, given glucose...	187	154	111	104
Postabsorptive, stimulated and recovering.................	113	103	66	70
Postabsorptive, stimulated and recovering, given glucose.................	203	156	120	116

[a] Values are averages of several results and expressed as counts per minute per milligram P calculated on the basis of one million counts per minute injected per kilogram body weight. Time of experiments, 90 minutes.

was slight in proportion to the simultaneously assimilated quantity of glucose. They therefore concluded that passage of glucose into the muscle cells in the form of hexose phosphate formed from the inorganic phosphate of the plasma was to be regarded as out of the question.

C. TURNOVER OF ACID-SOLUBLE PHOSPHORUS IN THE LIVER

The investigation of Kaplan and Greenberg[18] showed that the maximum ^{32}P content of the free phosphate of liver tissue of fasting rats reaches its maximum 75 minutes after intraperitoneal injection of labeled phosphate, while the maximum activity of the total acid-soluble phosphorus is obtained after a lapse of $110-120$ minutes (see Fig. 49).

The effect of dietary state of the animal on the distribution of acid-

[17] K. Kjerulf-Jensen and E. Lundsgaard, *Acta Physiol. Scand.*, **7**, 209 (1944).

[18] N. O. Kaplan and D. M. Greenberg, *J. Biol. Chem.*, **150**, 479 (1943); **156**, 511, 525 (1944).

soluble ^{31}P and ^{32}P in the liver both of animals receiving only ^{32}P and those receiving ^{32}P and 300–400 mg. glucose as well is seen in Table 95.

The total acid-soluble ^{32}P content of the liver is increased on all but the high-fat diet. In the fasted, fat-fed, and protein-fed rats a relatively large part of the ^{32}P is present in inorganic form. These animals show a decreased ability to fix inorganic phosphate. The liver of the high-carbohydrate-fed animals shows a decrease in inorganic ^{32}P content. These animals convert a larger percentage of the labeled phosphorus of the liver into organic form than do animals on any other diet.
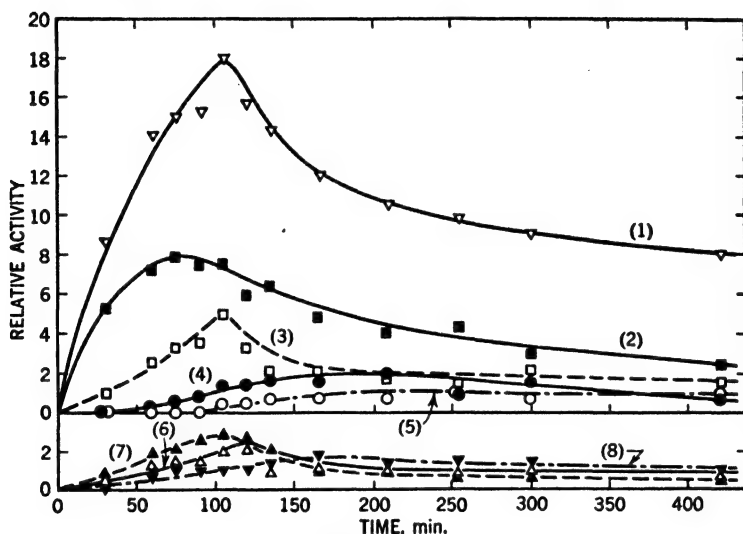
Fig. 49.   Distribution of ^{32}P in liver phosphorus fractions after administration of $Na_2H^{32}PO_4$. (1) total acid-soluble P; (2) inorganic P; (3) alcohol precipitate; (4) labile P in adenosine triphosphate; (5) nonlabile P in adenosine triphosphate; (6) mercury precipitate; (7) residual P; (8) lead precipitate. The points at 105 minutes are an average of ten determinations.

It is apparent from examination of the curves of Figures 49 and 50 that injection of glucose causes a marked change in time pattern of liver phosphate metabolism. In contrast to the control animals a great deal of ^{32}P is rapidly incorporated into the labile groups of adenosine triphosphate. Inorganic ^{32}P disappears more rapidly in the glucose-treated animals than in the controls. Inorganic ^{31}P also decreased as a result of introduction of glucose.

The effect of propanediol phosphate on the rate of renewal of the acid

TABLE 95. Effect of Dietary State of Animal on Distribution of Acid-Soluble 31P and 32P in Liver[18]

Diet[a]	No. of animals	Total acid soluble 31P	Total acid soluble 32P	Inorganic 31P	Inorganic 32P	Labile P of ATP 31P	Labile P of ATP 32P	Residual 31P	Residual 32P	Mercury ppt. 31P	Mercury ppt. 32P	Alcohol ppt. 31P	Alcohol ppt. 32P
						Animals receiving only 32P							
Controls (stock diet), fasted 12 hr	5	95.4 ±1.9	173.5 ±6.4	23.0 ±0.67	77.5 ±3.5	12.0 ±0.46	12.3 ±0.77	8.1 ±0.37	25.9 ±2.3	11.3 ±0.44	21.6 ±1.3	27.4 ±0.98	46.7 ±2.1
Fasted 72 hr	6	83.2 ±2.6	206.8 ±7.0	27.7 ±0.94	138.6 ±4.2	7.4 ±0.27	10.0 ±1.1	8.0 ±0.33	13.4 ±1.1	9.9 ±0.33	13.7 ±1.2	21.3 ±0.88	22.0 ±1.5
High carbohydrate	5	107.2 ±1.7	212.7 ±4.5	19.9 ±0.42	66.0 ±2.9	13.2 ±0.83	15.1 ±1.3	10.5 ±0.55	30.3 ±1.5	12.4 ±.048	28.7 ±2.0	31.4 ±1.2	53.9 ±2.9
High fat	5	80.6 ±2.9	143.2 ±7.2	22.9 ±0.66	88.6 ±3.4	5.7 ±0.51	10.1 ±1.2	8.8 ±0.46	18.1 ±1.5	8.8 ±0.55	15.3 ±1.4	21.9 ±0.67	26.5 ±2.4
High protein	5	86.0 ±0.79	204.4 ±5.9	30.6 ±0.60	119.6 ±4.3	8.6 ±0.23	10.7 ±0.63	9.5 ±0.65	25.2 ±3.2	8.8 ±0.50	20.1 ±1.7	21.8 ±1.1	30.6 ±2.0
						Animals receiving 32P and 400 mg. glucose							
Controls (stock diet)	5	103.4 ±2.0	206.6 ±5.5	20.0 ±0.72	58.3 ±3.3	20.8 ±0.45	46.5 ±2.3		13.4 ±1.2	11.2 ±0.74	21.0 ±0.80	29.7 ±1.2	27.9 ±2.1
Fasted 72 hr	5	85.1 ±1.8	208.5 ±8.3	26.5 ±1.0	116.6 ±7.0	12.9 ±0.75	24.4 ±1.3			9.1 ±0.39	18.5 ±1.2	21.6 ±0.96	18.6 ±1.2
High carbohydrate	5	115.4 ±2.2	241.3 ±6.8	19.4 ±0.75	58.6 ±2.3	22.3 ±0.59	57.7 ±2.5			13.5 ±0.98	33.0 ±1.5	34.4 ±1.3	32.5 ±2.1
High fat	4	84.5 ±2.0	152.5 ±6.2	20.9 ±0.64	75.4 ±3.3	10.5 ±0.52	22.1 ±0.89			9.7 ±0.87	21.3 ±2.3	21.7 ±0.67	18.6 ±1.4
High protein	5	87.9 ±1.2	218.9 ±4.2	27.0 ±1.2	101.4 ±4.3	14.5 ±0.62	30.3 ±2.1			9.0 ±0.71	25.9 ±1.8	22.2 ±0.86	27.5 ±2.0

a All animals except those fasted 72 hours were fasted 12 hours before injection with the Na₂HPO₄ containing 32P. All animals were sacrificed 110 minutes after receiving the injection. The values for 32P are in milligrams P per 100 grams fresh liver; 32P in parts per 1000 of the administered dose per 100 grams fresh liver. The measure of variability is the standard error of the mean.

soluble phosphorus fractions in rat liver was investigated by Lindberg.[19] For 20 days rats were fed a diet containing 100 mg. phosphorus as propanediol phosphate. To the rats weighing 55 g. labeled sodium phosphate was then injected intraperitoneally. After the lapse of one hour the acid-soluble phosphorus compounds of the liver were extracted. The specific activity of the phosphorus fraction obtained by acid hydrolysis for 10 to 20 minutes, the "hexose ester" fraction, was found to be much higher than that of the corresponding fraction in the controls.
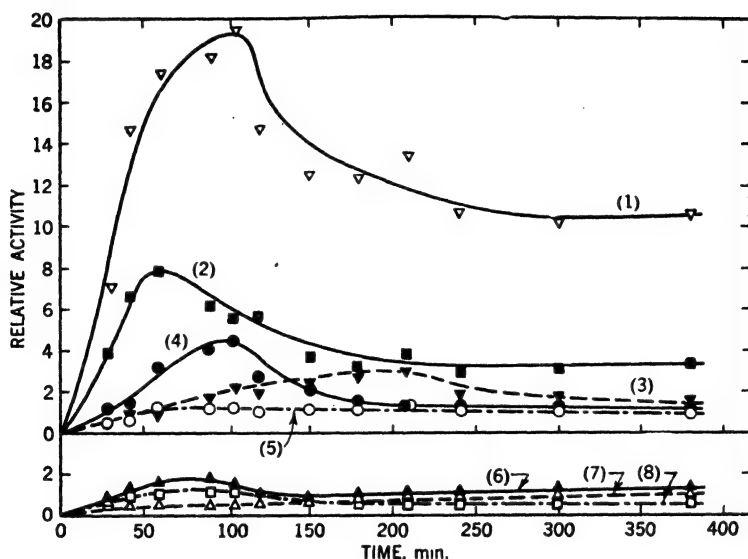
Fig. 50. Distribution of ^{32}P in acid-soluble phosphate fractions of livers of glucose-injected rats after $Na_2H^{32}PO_4$ administration. Identity of the curves is the same as in Figure 49.

In tissue slices of rat kidney and liver the accumulation of organic ^{32}P was found to increase appreciably when sodium fluoride was added to the medium containing labeled phosphate. The ^{32}P was found to be present in the phosphoglyceric acid fraction. In the absence of oxygen accumulation of ^{32}P in the organic fractions was found to be much reduced.[20]

Effect of Insulin on the Phosphorus Turnover in Liver. Insulin and glucose administrations have a marked influence on acid-soluble ^{32}P

[19] O. Lindberg, *Arkiv Kemi Mineral. Geol.*, **A23**, No. 2 (1946).

[20] N. O. Kaplan, I. Memelsdorf, E. Dodge, *J. Biol. Chem.*, **160**, 631 (1945).

content of liver.[18] Rats weighing about 200 g. were fasted for a period of twelve hours, given the treatment shown in the table, and then injected intraperitoneally with trace doses of labeled sodium phosphate. The results obtained are seen in Table 96.

TABLE 96

Distribution[a] of Labeled Phosphorus in Acid-Soluble Phosphorus of Liver 110 Minutes after Distribution of the Phosphate[18]

Treatment	Total acid soluble	Inorganic	ATP		Alcohol soluble[b]	Residual[c]
			Labile	Nonlabile		
Control, fasted 12 hr.	177	77.6	11.7	3.3	44.0	28.6
Glucose (300–400 mg. intraperitoneally).....	204.0	56.6	49.0	12.8	25.4	12.9
Insulin	244.3	92.5	41.5	15.6	26.2	14.5
Glucose and insulin.....	262.0	83.4	64.5	16.2	22.7	20.2

[a] All values are in per cent of administered dose × 10 of the labeled P per 100 grams fresh liver.
[b] This fraction consists largely of glycerol phosphate, but it contains also some hexose monophosphates.
[c] This fraction consists largely of phosphoglyceric acid.

The maximum in ^{32}P concentration of the total acid-soluble phosphates was usually attained at about 110 to 120 minutes (see Figs. 49 and 50). The peak in radioactivity of the total phosphorus of ATP in the animals given glucose occurred at 110 minutes after the injection of ^{32}P, whereas the peak in the control group occurred at 200 minutes. The peak in radioactivity of nonlabile phosphorus of the adenosine triphosphate was similarly shifted from 250 to 110 minutes. The peaks in the radioactivity of the alcohol and residual fraction were displaced in time by glucose administration from 110 to 210 and 245 minutes, respectively.

Phlorizin, malonate, and fluoride, but not iodoacetate, prevent a rise in the ^{32}P content of liver following administration of glucose. These findings support the hypothesis that the primary action of phlorizin is a blocking of the formation of ATP.

Reduction of food intake causes a decrease in the acid-soluble phosphorus content of liver.[21] The effect of insulin may therefore possibly partly or wholly be due, not to an increased rate of renewal of acid-soluble phosphorus molecules present in the liver, but to an increase in total ATP and inorganic phosphate content following the administration of insulin. In the investigations described above the labile phos-

[21] N. Nelson, S. Rapoport, G. M. Guest, and I. A. Mirsky, *J. Biol. Chem.*, **144**, 291 (1942).

phorus of ATP is identified as the "seven minutes acid-soluble phosphorus." Objections may possibly be raised to this identification.[21a]

In experiments on perfused cat liver, a high percentage of the ATP molecules and a minor percentage of the ester phosphorus were found by Lundsgaard to be renewed in the course of one hour.[22]

Five minutes after intravenous injection of radioactive phosphate, Kalckar and his associates[23] found the labile phosphorus of adenyl pyrophosphate present in rabbit liver to have a specific activity of 83% of the corresponding value for inorganic intracellular phosphorus. In interpreting this and similar figures, we must take into account that in the course of five minutes the plasma activity declines considerably, very highly active phosphate penetrating the liver cells in an early phase of the experiment. When we calculate the percentage renewal from the ratio of the specific activity of the pyrophosphate phosphorus and that of the inorganic phosphorus at the end of the experiment, we obtain correspondingly too high a figure for the extent of renewal. Kalckar and associates estimate the rate of rejuvenation of pyrophosphate phosphorus as $15 - 20$ μg. per minute per gram liver and consider these to be minimum values.

Rapoport and colleagues[24] found the rate of renewal of labile phosphorus and adenosine triphosphate of kidneys to be reduced under the action of phlorizin. While in the controls the rate of renewal of labile phosphorus amounted to 70% in thirty minutes when phlorizin was previously administered by intravenous injection, the value was reduced to 33%.

D. EFFECT OF INSULIN ON TURNOVER OF ACID-SOLUBLE PHOSPHORUS

Kaplan and Greenberg[25] investigated the phosphate changes in blood, muscle, and liver, following intravenous administration of insulin. Labeled phosphate and in some of the experiments 0.75 I. U. insulin per kilogram body weight were administered to rabbits. After the lapse of 90 minutes the animals were sacrificed and the specific activities of the total acid-soluble, the inorganic, and the barium-soluble phosphorus

[21a] H. M. Kalckar, *Ann. Rev. Biochem.*, **14**, 283 (1945).

[22] E. Lundsgaard, *Skand. Arch. Physiol.*, **80**, 291 (1938).

[23] H. M. Kalckar, *J. Biol. Chem.*, **154**, 267 (1944). H. M. Kalckar, J. Dehlinger, and A. Mehler, *ibid.*, **154**, 275 (1944).

[24] S. Rapoport, N. Nelson, G. M. Guest, and I. A. Mirsky, *Science*, **93**, 88 (1941).

[25] N. O. Kaplan and D. M. Greenberg, *Am. J. Physiol.*, **140**, 598 (1944).

extracted from the blood, muscle, and liver were determined. As is seen in Table 97, insulin causes an increase in the total acid-soluble ^{32}P of the liver and muscle; an increase in the total acid-soluble ^{31}P was noted only in the liver. Inorganic ^{32}P leaves the blood at a more rapid rate than ^{31}P. Both the ^{31}P and ^{32}P contents of the barium-soluble fraction of muscle and liver increase due to the action of insulin.

TABLE 97

Effect of Insulin on Distribution[a] of ^{31}P and ^{32}P in Blood, Muscle, and Liver of the Rabbit[25]

Rabbit	Total acid-soluble			Inorganic			Barium-soluble		
	^{31}P	^{32}P	Specific activity	^{31}P	^{32}P	Specific activity	^{31}P	^{32}P	Specific activity
Blood									
Control......	51.3	122.4	2.40	5.0	64.0	12.8	4.3	11.8	2.80
Insulin-treated	54.3	103.1	1.87	3.6	35.4	9.82	8.5	21.1	2.57
Muscle									
Control......	139.7	68.1	0.49	92.1	52.2	0.57	14.0	7.4	0.52
Insulin-treated	141.8	85.0	0.60	82.0	38.2	0.47	27.5	27.6	0.98
Liver									
Control......	79.0	215.9	2.72	19.1	71.1	3.74	37.4	64.7	1.72
Insulin-treated	94.6	297,1	3.14	24.6	87.4	3.55	42.4	90.1	2.19

[a] Values for ^{31}P are given in milligrams per 100 grams fresh tissue.

Weissberger[26] investigated the influence of insulin, epinephrine, and glucose administration on several phosphorus fractions of the blood of guinea pigs. The specific activities of the barium-soluble and of the alcohol-insoluble phosphorus were not found to be influenced.

E. EXPERIMENTS *IN VITRO* ON RATE OF RENEWAL OF ADENOSINE TRIPHOSPHATE

Experiments *in vitro*, in which much simpler conditions prevail than *in vivo*, were used for a calculation of the rate of renewal of the labile phosphorus in ATP. In determining the rate of interchange between

[26] L. H. Weissberger, *J. Biol. Chem.*, **160**, 48 (1945).

free phosphate and pyrophosphate, Meyerhof *et al.*[27] studied the reaction: 2 triosephosphoric acid + 2 pyruvic acid + 1 adenosine-monophosphoric acid + 2 phosphoric acid = 2 phosphoglyceric acid + 2 lactic acid + 1 adenosinetriphosphoric acid.

To a solution, kept at 20°C., containing dialyzed muscle extract, phosphoglyceric acid, hexose diphosphate, sodium lactate, pyruvic acid, adenosine triphosphate (containing 0.327 mg. pyrophosphate phosphorus), magnesium, manganese, sodium fluoride, and cozymase, labeled phosphate (containing 0.395 mg. phosphorus) was added. The distribution of labeled atoms between the free phosphate and the pyrophosphate fraction was then determined at different intervals. After the lapse of twenty seconds, as is seen in Table 98, almost half of exchange equilibrium of ^{32}P between the two fractions was obtained. The average time of interchange of a pyrophosphate group with a phosphate group was shown to be 50 seconds.

It is quite possible that in muscle cells, where the enzyme concentration is ten times as large as in the experiments described above, a similar or even more rapid interchange takes place. The observation — that *in vivo*, after a lapse of several minutes, the interchange is somewhat below 100% (see page 256) — may be due to obstacles the labeled phosphate ions must overcome to reach the place of formation of ATP molecules. But it is also possible that the average rate of renewal of the ATP molecules in cells is smaller than that found in the system described above. Whether all ATP molecules present in the muscle cells behave in the same way — and the same question applies to other compounds as well — is a problem in the solution of which isotopic indicators may play an important part.

TABLE 98

Distribution between Inorganic and Pyrophosphate P of 0.395 Milligram Labeled Inorganic P Added to an *in Vitro* Muscle System[27]

Time, sec.	Inorganic P, mg.	Pyrophosphate P, mg.	Interchange, %
0	0.395	0	0
20	0.313	0.084	47
40	0.288	0.120	64
75	0.253	0.135	77
150	0.255	0.144	85
600	0.223	0.159	92
	0.216	0.179	100

[27] O. Meyerhof, P. Ohlmeyer, W. Gentner, and H. Maier-Liebnitz, *Biochem. Z.*, **298**, 396 (1938).

The renewal of phosphorus compounds goes hand in hand with the phosphorylation processes that are closely connected with oxidative steps in the utilization of carbohydrates. Each phosphorylation first involves adenosine phosphate and thus necessitates a renewal of the labile phosphorus of ATP molecules. Assuming two phosphorylations through the terminal group of ATP for every atom of oxygen, and taking the oxygen consumption to be 1.0 ml. per g. per hour, Furchgott and Shorr[28] calculate that, in 1 g. of cardiac tissue of the dog, 178.4 micromoles of phosphorylation takes place. This would represent a turnover of phosphorus, from intracellular inorganic P to the terminal group of ATP, of about 92 μg. per g. tissue per minute. Assuming a rate of renewal in the resting muscle corresponding to that found by Meyerhof and associates in their experiments *in vitro*, and taking 1 g. muscle to contain 120 μg. terminal ATP phosphorus, the rate of turnover proves to be 144 μg. per g. tissue per minute.

Meyerhof[29] arrived at the result that the glucose molecule in yeast passes anaerobically through twelve stable intermediary stages before becoming alcohol and CO_2 (glucose metabolism in *Fusarium* is reported to show a different behavior).[30] At least three dissociable organic coenzymes, twenty or more enzyme proteins, and some bivalent metals (manganese and magnesium) are regarded as necessary to the breakdown.[29] Since phosphate participates in almost every reaction, the application of labeled phosphorus in a detailed study of glucose metabolism may elicit information of interest. While, as described above, radiophosphorus was applied in the study of some phases of glucose metabolism, an extended application of this tracer in such studies has as yet not been made.

F. EFFECT OF HYPERTHYROIDISM ON TURNOVER RATE

Tracer experiments were performed with radioactive phosphorus by Greenberg et al.[31] on the turnover of acid-soluble phosphates to study the role of the thyroid in carbohydrate metabolism. It was observed that hyperthyroid activity affected the rate of turnover of ^{32}P in muscle,

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[28] R. F. Furchgott and E. Shorr, *J. Biol. Chem.*, **151**, 65 (1943).

[29] O. Meyerhof, *J. Biol. Chem.*, **157**, 105 (1945).

[30] F. F. Nord and R. P. Mull, in *Advances in Enzymology*, Vol. V. Interscience, New York, 1945.

[31] D. M. Greenberg, J. Fraenkel-Conrat, and M. B. Glendening, *Federation Proc.*, **6**, No. 1 (1947).

liver, and kidney with little or no associated alterations in the total acid-soluble, inorganic, or labile phosphorus fractions. The ^{32}P content of the total acid-soluble, inorganic, and labile phosphorus fractions of liver and kidney was 25 to 50% lower, while that of muscle was over 100% greater, 110 minutes after administration, in hyperthyroid than in fasted control rats. In the blood serum of the hyperthyroid rats the inorganic phosphate was increased but the ^{32}P was decreased.

In hypothyroidism it was found that the accumulation of ^{32}P was increased in blood, liver, and kidney but decreased in muscle. Comparison of the ratios of the specific activities of the phosphorus fractions of the three tissues to that of the blood showed that liver had the greatest and muscle the lowest ability to concentrate phosphorus. There was, however, little difference between the liver and kidney in hyperthyroid and control animals in this respect. On the other hand, the ratio of specific activities was much higher for the muscle of the

TABLE 99

Specific Activities[a] of Total Phosphorus of Brain Areas and Blood of Rats [32]

Part	Interval between injection of ^{32}P and killing of animal						
	40 min.	90 min.	2 hr.	2 hr., 20 min.	2 hr., 45 min.	24 hr.	24 hr.
Pineal body...............	820	520	1710	775	2080	...	...
Lobus anterior hypophysis...	258	410	600	565	652	390	640
Lobus posterior hypophysis..	234	355	290	492	440	250	425
Plexius chorioideus.........	930	615	975	1040	420	485	255
Lobus olfactorius...........	90	124	180	125	200	136	...
Lobus occipitalis...........	70	72	150	86	93	92	...
Lobus parietalis............	78	106	60	43	93	...	...
Thalamus.................	42	75	45	43	...	32	100
Anterior tuber cinereum.....	136	138	110	146	185	97	100
Posterior part of tuber cinereum...................	63	69	115	82	180	97	100
Corpus mamillare..........	50	...	95	111	140	80	142
Substantia perforata........	63	...	85	68	233	59	325
Corpora quadrigemina......	63	...	65	71	100	40	115
Cerebellum................	100	100	100	100	100	100	100
Pons.....................	63	56	45	50	...	54	100
Medulla oblongata.........	48	73	72	64	...	97	85
Blood....................	4350	2920	1910	2800	2550	880	410

[a] Specific activity of cerebellum = 100.

hyperthyroid animals, evidently due to a more rapid uptake of ^{32}P. The more rapid uptake of ^{32}P by muscle in hyperthyroidism may serve to explain the relative deficiency observed in the blood, liver, and kidney. The observed relationships suggest that thyroid activity influences the rate of transfer of phosphorus across cell membranes.

G. PHOSPHORUS METABOLISM IN BRAIN

Borell and Örström[32] investigated the uptake of ^{32}P by different parts of the brain. The phosphorus present in the pineal body, the anterior and posterior lobes of the hypophysis, and the plexus chorioideus was found to interchange with the labeled phosphate administered by intraperitoneal injection at a much more rapid rate than the other parts of the brain (see page 293); the latter exhibit about the same slow phosphorus interchange as the cerebellum, as seen in Table 99.

Of the ^{32}P accumulated in the course of 40 minutes in the pineal body about 65% are present as acid-soluble phosphorus, 25% as inorganic phosphorus, and 10% as nonacid-soluble phosphorus. After castration, increased ^{32}P uptake was observed in the pineal body and anterior pituitary. This increase is most pronounced in females.

H. APPLICATION OF RADIOPHOSPHORUS IN STUDIES ON METABOLISM OF THE SEXUAL CYCLE

Radioactive phosphorus was used by Borell and co-workers[33] in the study of metabolic processes taking place in the tuber cinereum, adenohypophysis (anterior lobe of pituitary), and ovaries in the different phases of the sexual cycle of the rabbit. Labeled sodium phosphate in 5% glucose solution was injected into the veins of anoestrous and oestrous female rabbits, either before or after mating. To each animal about 0.1 millicurie ^{32}P was administered. After the lapse of 30 minutes the rabbit was killed by decapitation.

Mating was found to increase very substantially the uptake of ^{32}P by the tuber cinereum and to a minor extent by the adenohypophysis of the rabbit, as seen in Table 100. In the tuber cinereum the increased activity is maintained during the first hour after mating. Subsequently, it decreases to values approaching those found in oestrous animals. In the adenohypophysis the high ^{32}P content is maintained during the

[32] U. Borell and Å. Örström, *Acta Physiol. Scand.*, **10,** 231 (1945); *ibid., in press.*

[33] U. Borell, A. Westman, and Å. Örström, *Gynaecologia*, **123,** 186 (1947).

whole observation time, *viz.*, until 24 hours after mating, with a peak in the second half-hour after mating.

In contradistinction to the above-mentioned organs, in the ovaries there is no increase in ^{32}P until 30 minutes after mating. The activity is then maintained at a comparatively high level and reaches another maximum about 9 – 11 hours after mating, *i.e.*, when ovulation in the rabbit occurs.

TABLE 100

Specific Activity of Total ^{32}P in Different Organs of the Rabbit[33]

Group of rabbits	Tuber cinereum	Adenohypophysis	Ovaries
Anoestrous.....................	2.60	28.20	25.14
Oestrous control................	2.98	30.26	28.05
Mating 2 min. before decapitation.	4.65	38.40	
Mating 5 min. before decapitation.	4.95	43.00	
Mating 10 min. before decapitation.	5.55	46.70	
Injected immediately after mating .	4.92	45.00	27.00
Injected 30 min. after mating......	5.18	51.20	54.40
Injected 60 min. to 24 hr. after mating	3.28	42.50	55.00
Castrated animals...............	2.78	49.70	

No substantial difference is observed in the ^{32}P uptake by the anoestrous and oestrous rabbits.

That the enhanced uptake of ^{32}P after mating is not due simply to

TABLE 101

Effect of Mating on Rate of Formation of Organic P Fraction
of Different Organs Obtained after Ten-Minute
Hydrolysis of Acid-Soluble Compounds[33]

Organ	Group of rabbits	Specific activity		
		Inorganic P	Organic acid-soluble P (10-min. product)	Total acid-soluble P
Tuber cinereum	Anoestrous	2.97	2.28	0.19
	Mated	2.50	9.45	0.30
Adenohypophysis	Anoestrous	17.30	8.95	0.50
	Mated	31.90	16.50	0.65
Ovaries	Anoestrous	27.60	3.25	3.11
	Mated	41.80	8.30	2.62
Cerebellum	Anoestrous	1.15	1.47	1.72
	Mated	0.85	1.29	1.60

increased permeability of the organs investigated is suggested by results obtained when comparing the specific activity of an organic phosphorus fraction of an organ with the specific activity of the inorganic phosphorus fraction of the same organ. The organic phosphorus fraction was obtained by acid hydrolysis for 30 minutes of the trichloroacetic acid filtrate secured by extracting the organ. The ratio of the specific activities of the organic and the inorganic phosphorus in the case of tuber cinereum is very markedly increased (from 0.72 to 3.8) after mating, as seen in Table 101. In these experiments, ^{32}P was administered 30 minutes after mating, and the rabbit was killed 30 minutes later. When interpreting the results of these short-time experiments, it must be taken into account that an appreciable part of tissue inorganic ^{32}P may be of extracellular origin (see page 255).

The investigations as a whole furnish further evidence that the hypophyseal – diencephalic system constitutes a functional unit which, by its stimulating and regulating action, plays a controlling part in the process of ovulation.

III. Turnover of Phosphatides

A. GENERAL REMARKS

As was shown by early experiments, the presence of labeled phosphatides can be detected in the tissues shortly after administration of labeled phosphate.[33a-35d] The percentage of the dose administered present in the liver of the rat as phosphatide (^{32}P) first increases, then declines after about ten hours, as is seen in Figures 51 to 55 taken from a publication of Perlman and associates.[35] A similar behavior is shown by the labeled phosphatide content of the gastrointestinal tract. The labeled phosphatide content of the carcass increases up to 100 hours.

The rapid increase in labeled phosphatide content of the liver indicates rapid turnover. In the early phases of the experiment, the specific

[33a] L. Hahn and G. Hevesy, *Skand. Arch. Physiol.*, **77**, 148 (1937).

[34] C. Artom, G. Sarzana, M. Santangelo, and E. Segrè, *Arch. intern. physiol.*, **45**, 32 (1937).

[35] I. Perlman, S. Ruben, and I. L. Chaikoff, *J. Biol. Chem.*, **122**, 169 (1937).

[35a] C. Artom, G. Sarzana, and E. Segrè, *Arch. intern. physiol.*, **47**, 245 (1938).

[35b] B. A. Fries, S. Ruben, I. Perlman, and I. L. Chaikoff, *J. Biol. Chem.*, **123**, 587 (1938).

[35c] I. L. Chaikoff, *Physiol. Revs.*, **22**, 291 (1942).

[35d] C. Artom, G. Sarzana, C. Perrier, M. Santangelo, and E. Segrè, *Nature*, **139**, 836 (1937).

activity of cellular phosphate is high and the newly synthesized phosphatide molecules incorporate highly active phosphate. The labeled
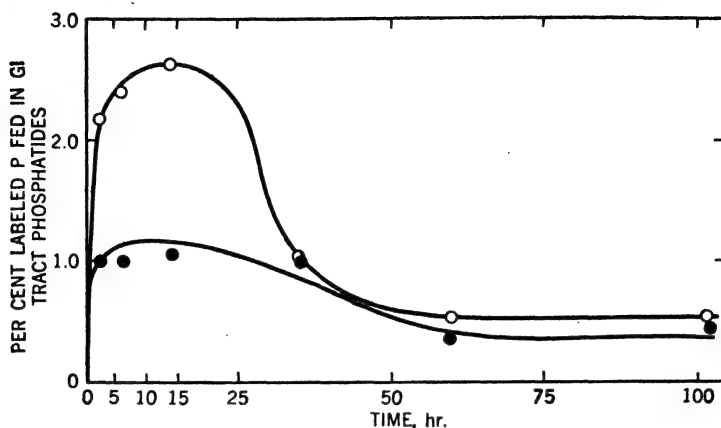
Fig. 51. Labeled phosphatide content of the gastrointestinal tract[35] obtained from rats fed 1 cc. cod-liver oil along with 4 mg. of labeled P (O) and from rats that received 4 mg. of labeled P (●). Each point represents the average of four analyses on two rats.
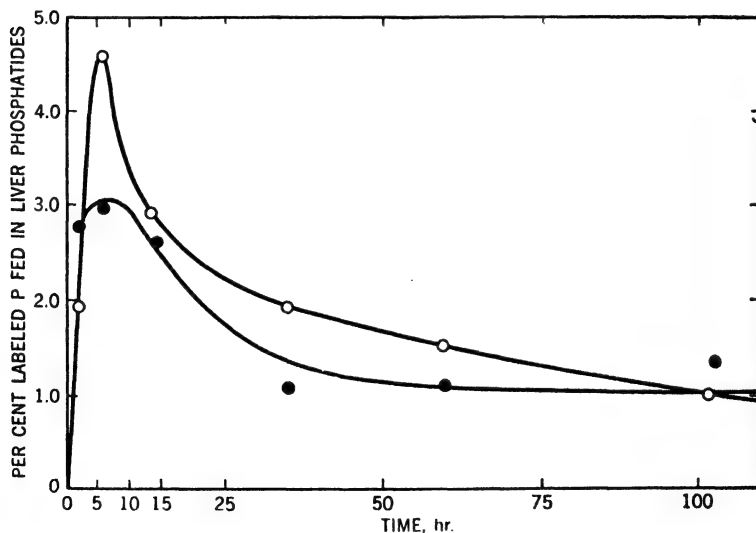
Fig. 52. Labeled phosphatide content of liver.[35] Symbols have the same meaning as in Figure 51.

phosphate molecules in later phases of the experiment are repeatedly renewed with the participation of less active phosphate, and labeled

phosphate makes its way in the later phases of the experiment from the liver into the plasma.   This leads to a decrease in labeled phosphatide
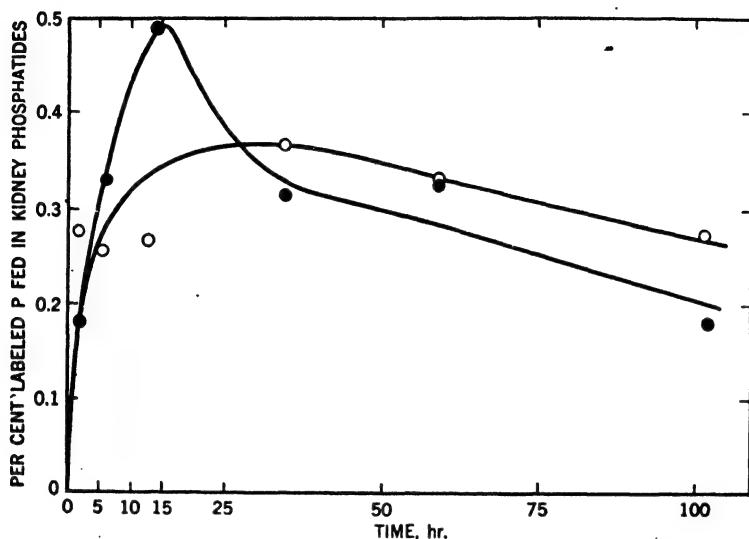
Fig. 53.   Labeled phosphatide content of kidney.[35]   Symbols have the same meaning as in Figure 51.
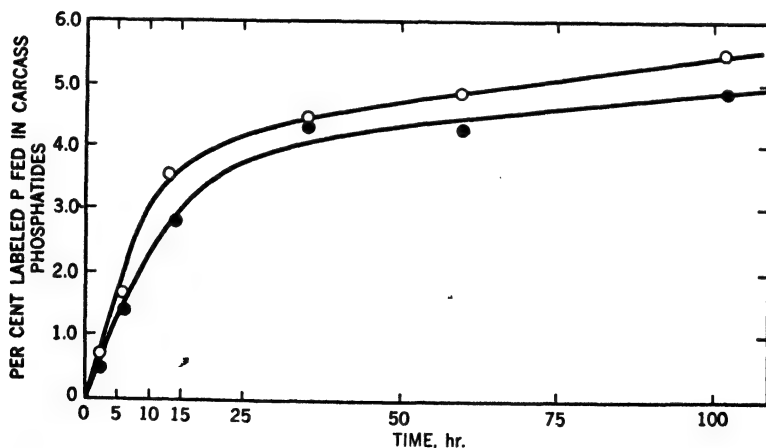
Fig. 54.   Labeled phosphatide content of carcass.[35]   Symbols have the same meaning as in Figure 51.

content of the liver.   Apart from the high rate of renewal of phosphatide molecules, a high permeability of the liver cells to phosphate is responsible for the shape of the curve shown in Figure 52.   Labeled phospha-

tides of the liver, furthermore, can enter the circulation, but the amount leaving the liver is only 1% of the phosphatide content of the liver per hour (see page 291). That the labeled phosphatide content of the muscles increases in the course of the first 100 hours (see Figure 54) may be interpreted as due partly to the low rate of formation of labeled phosphatide molecules in the muscle or alternatively to the low rate of penetration of phosphatides into the muscle cell (see page 304).
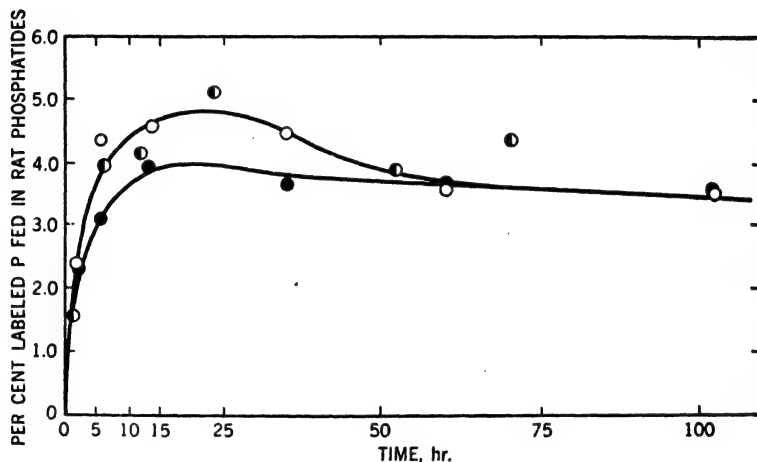
Fig. 55. Labeled phosphatide content of the whole rat[35] obtained from rats fed 1 cc. cod-liver oil along with 1.5 mg. labeled P (◑), from rats fed 1 cc. of cod-liver oil along with 4 mg. of labeled P (O) and from rats fed 4 mg. of labeled P only (●). All points obtained by summation of the results of analyses of individual tissues.

The above data indicate the formation of a labeled phosphatide mixture mainly composed of lecithins, cephalins, and sphingomyelins. The rates of formation of these constituents of the phosphatides vary, though the differences are, in most cases, as discussed on page 279, not very pronounced. The maximum activity of the total labeled phosphatide content of the rat is reached after the lapse of about 24 hours; while in the liver the most active organ, a maximum content is obtained about 10 hours after administration of labeled phosphate.

In the fasting mouse, Hodge and associates[36] found the specific

[36] H. C. Hodge, P. L. MacLachlan, W. R. Bloor, E. A. Welch, and S. Levy, *Federation Proc.*, **2**, 63 (1943). P. L. MacLachlan, H. C. Hodge, W. R. Bloor, E. A. Welch, F. L. Truax, and J. D. Taylor, *J. Biol. Chem.*, **143**, 473 (1942).

activity of all phosphatide fractions, with the exception of the α-cephalin fraction, to increase appreciably. A maximum specific activity for the α-cephalin was reached on the second fasting day.

The total amount of ^{32}P incorporated in the phosphatides of normal and rachitic rats was determined by Dols and associates.[36a] Rachitic rats were found to contain less total ^{32}P but more phosphatide ^{32}P than the controls investigated. The phosphatide phosphorus content of normal rats weighing $35-40$ g. was found to be 11% of their total phosphorus content.

The ultimate aim of investigation of the origin of phosphatide molecules in the body is the determination of the form in which the hydrogen carbon, nitrogen, oxygen, and phosphorus atoms of phosphatide molecules have been taken up by the body and of the steps in which these atoms have been involved before their ultimate incorporation into phosphatide molecules. This exacting problem can hardly be solved at present, and we must be content with the determination of the sites and rates of formation of phosphatide molecules in the body from glycerol, fatty acid, choline (or other organic bases), and phosphate.

The lecithin molecule may be regarded as formed from five primary components: two fatty acids, glycerol, choline, and phosphoric acid. These are joined by two general types of bonds: the fatty acid-glycerol ester linkage and the phosphate–alcohol ester bond. Phosphate is bound in the lecithin molecule by two ester linkages, one of which is with glycerol and the other, with the base choline. It is conceivable that, after the phosphate group is split off the phosphatide enzymically, other ("new") phosphate radicals become combined with the dephosphorylated compound at a more rapid rate than the renewal of this compound takes place. In this instance the rate of renewal of the phosphate radicals will be greater than the rate of renewal of the other components of the molecule, and the turnover rate of phosphatide phosphate will supersede that of the fatty acids and nitrogen base present in the molecule. A combined investigation of the turnover rate of phosphatides, using labeled phosphorus, labeled nitrogen, and labeled hydrogen may be expected to supply information regarding the relative rates of renewal of the different constituents of the phosphatide molecule.

If we make the assumption that phosphate ions or phosphorus-containing precursors which attain rapid equilibrium with the phosphate

[36a] M. J. L. Dols, B. C. P. Jansen, G. J. Sizoo, and F. Barendregt, *Proc. Acad. Sci. Amsterdam*, **41**, 997 (1938); **42**, 2 (1939).

ions are incorporated into the phosphatide molecule, we can determine the rate of renewal of phosphatide molecules by comparing the specific activity of the phosphatide phosphorus at the end of the experiment with the mean specific activity of the intracellular inorganic phosphorus prevailing during the experiment. This calculation involves the further assumption that it is the intracellular, inorganic phosphorus which is incorporated into the phosphatide molecule. The rate of renewal of phosphatides extracted from the liver of the rat and of the rabbit is calculated by comparing the specific activities of the phosphatide phosphorus and tissue inorganic phosphorus at the end of the experiment taking four hours, the result obtained being shown in Table 102.[37] Since the average specific activity of cellular inorganic phosphorus of the liver in experiments taking four hours does not differ very much from the specific activity of cellular inorganic phosphorus measured at the end of the experiment[38] and the difference between the specific activities of the intracellular and tissue inorganic phosphorus is in this case negligible, when computing the data of Table 102 the tissue inorganic phosphorus values determined at the end of the experiment were considered.

TABLE 102

Renewal of Lecithin and Cephalin in Rat and Rabbit Liver[37]

Fraction	Per cent renewal[a]	
	Rat liver	Rabbit liver
Free P.............	100	100
Lecithin P..........	20.4	6.3
Cephalin P..........	18.6	6.0

[a] Radiophosphorus administered four hours prior to killing the animal.

The knowledge of exact turnover figures is of interest when comparing, for example, the amount of fat metabolized with the amount of phosphatides turned over. In many cases however we can draw conclusions merely by comparing the specific activity of the phosphatides present in an organ under varying conditions. Such a procedure is applied in the study of the effect of ingested fat on the rate of renewal of phosphatides, of the effect of choline, cholesterol, and other sub-

[37] L. Hahn and H. Tyrén, *Arkiv Kemi Mineral. Geol.*, **A21**, No. 11 (1946).
[38] L. Ahlström, H. v. Euler, and G. Hevesy, *Arkiv Kemi Mineral. Geol.*, **A19**, No. 9 (1944).

stances on the turnover of liver phosphatides, of the site of formation of the yolk, embryo, and milk phosphatides, and so on.

A comparison of turnover rate of phosphatides and of other compounds, not only in different organs but even in different parts of cells, is a problem of great importance, solved so far only in a special case: the rate of renewal of cytoplasm phosphatides has been compared with the rate of renewal of nuclear phosphatides, as described on page 341.

B. PURIFICATION OF PHOSPHATIDES

After administration of labeled phosphate free phosphate and also some of the acid-soluble fractions of organs are often much more active than the phosphatide fraction. It is therefore of great importance to purify the phosphatides and free them from all other active fractions. Levene's classical method, which is based on the extraction of the mineral constituents by shaking an ether solution of phosphatides with a water solution of acetic acid, does not remove the labeled free phosphate. When, however, acetic acid is replaced by hydrochloric acid, an effective separation can be obtained.[37] The effectiveness of this method of purification is seen from the figures of Table 102.[39]

TABLE 103

Purification of Solutions of Phosphatides in Ether with Inorganic ^{32}P Added[39]

Number of purifications[a]	Activity of ether solution	Activity of water solution
0	10,000	———
1	———	8600
2	———	60.4
3	———	8.8
4	———	2.6
5	5.5	2.0

[a] 100 ml. ether solution shaken repeatedly with 300 ml. 0.1 N HCl solution for 20 minutes.

Shaking the ether solution of the phosphatides with ten drops of saturated disodium phosphate solution containing excess solid disodium phosphate was found also to be very effective in removing traces of radioactive phosphorus.[40]

Separation of Choline-Containing and Non-choline-Containing Phosphatides

Taurog and co-workers[41] found that phosphatides are absorbed quantitatively from a petroleum ether solution by magnesium oxide;

[39] M. Anderson, *personal communication*.

[40] B. A. Fries, H. Schachner, and I. L. Chaikoff, *J. Biol. Chem.*, **144**, 59 (1942).

[41] A. Taurog, C. Entenman, A. Fries, and I. L. Chaikoff, *J. Biol. Chem.*, **155**, 19 (1944).

0.6 g. magnesium oxide suffices to absorb 0.85 mg. of phosphatide phosphorus dissolved in 15 ml. petroleum ether. Use of 1.75 g. magnesium oxide is however preferable when choline-containing phosphatides have to be separated from non-choline-containing phosphatides. The separation is based on the elution of the choline-containing phosphatides by methyl alcohol.

The separation of choline-containing phosphatides from non-choline-containing phosphatides can however also be accomplished by first dissolving the phosphatides in methanol and then treating this methanol solution with magnesium oxide. The following procedure is used.

Extraction of Phosphatides from Liver. The weighed tissue was ground with sand in a mortar, transferred to a flask, and extracted with alcohol at 55–60°C. for 2 hours with occasional shaking. Approximately 150 cc. of alcohol was used for 20–25 g. of liver. The supernatant alcohol was decanted through filter paper and the residue extracted with a second portion of alcohol for 1 hour. The contents of the flask were then poured through the same filter paper and the two alcohol extracts were combined. The tissue residue was then extracted overnight with ether in a Soxhlet apparatus and the ether extract was added to the alcohol extracts. The combined alcohol-ether extracts were concentrated to a small volume (3–4 ml.). This was carried out in a hot water bath (55–60°) under reduced pressure and in an atmosphere of carbon dioxide. This concentrate was extracted with several portions of petroleum ether (b.p. 30–60°) and the petroleum ether extract made up to a desired volume by the addition of more petroleum ether.

A measured volume of a petroleum ether extract of liver containing approximately 6 mg. of phosphatide phosphorus was evaporated just to dryness under reduced pressure in a carbon dioxide atmosphere and the residue was dissolved in methanol. Under these conditions all phosphatides were dissolved by the methanol. This was shown by phosphorus and choline determinations. The methanol solution was made to 100 ml., and 25-ml. aliquots containing approximately 0.060 mg. phosphatide phosphorus per milliliter were added to 1.75 g. magnesium oxide in 50-ml. centrifuge tubes. The mixtures were allowed to stand for 25–30 minutes with frequent stirring and then were centrifuged. The methanol was poured off and the magnesium oxide was washed twice with 25-ml. portions of fresh methanol. The combined methanol supernatants were made up to 100 ml. and aliquots were taken for phosphorus and choline determinations. The results are recorded in Table 104, which shows that the separation by this procedure is just as complete as when the phosphatides are first absorbed from a petroleum ether solution and then eluted with methanol.

One of the difficulties encountered was a cloudiness of the methanol solutions which appeared after centrifuging the magnesium oxide–methanol mixtures. The first methanol supernatant was usually clear, but the supernatants from the two methanol washings were usually cloudy. It was found that the presence of a small amount of sodium chloride in the methanol prevented the solution from becoming

turbid after centrifugation and did not affect the recovery of choline or the choline-phosphorus ratio. For this reason methanol containing approximately 5×10^{-3} M sodium chloride is used for the two washings of the magnesium oxide.

TABLE 104

Adsorption of Liver Phosphatides Directly from Methanol[41]

MgO used for adsorption, mg.	Phosphatide added to MgO			Phosphatide remaining in methanol			
	Choline, mg.	P, mg.	$\dfrac{\text{Moles choline}}{\text{moles P}}$	Choline, mg.	P, mg.	$\dfrac{\text{Moles choline}}{\text{moles P}}$	Choline phosphatide, %
Rat liver phosphatide							
1.0	3.51	1.51	0.59	3.49	0.99	0.90	99
1.75	3.42	1.49	0.59	3.36	0.88	0.98	98
1.75	3.42	1.49	0.59	3.25	0.85	0.98	95
1.75	3.42	1.49	0.59	3.30	0.91	0.93	96
Dog liver phosphatide							
1.5	3.56	1.55	0.59	3.38	0.93	0.93	95
1.75	3.56	1.55	0.59	3.22	0.86	0.96	90
1.75	3.56	1.55	0.59	3.25	0.88	0.95	91
1.75	3.46	1.51	0.59	3.14	0.82	0.98	91
1.75	3.46	1.51	0.59	3.13	0.82	0.98	91

C. EFFECT OF INGESTED FAT ON RATE OF RENEWAL

Some time ago Artom and colleagues[42] showed that feeding of oil promotes the turnover of phosphatides. Some of their results are recorded in Table 105.

The feeding of oil had the greatest effect on phosphatide metabolism in intestinal mucosa. A marked effect was also found in liver and kidney, but not in other organs of the rat. The increased phosphatide activity may be due either to the formation of additional phosphatide originating from the ingestion of oil, or to an accelerated rate of replacement of the nonactive phosphatide molecules by newly formed active molecules, or to both of these effects.

The effect of ingested fat on the activity of tissue phosphatides has also been investigated by Perlman and associates.[43] They studied

[42] C. Artom, G. Sarzana, and E. Segrè, *Arch. intern. physiol.*, **47**, 245 (1938).
[43] I. Perlman, S. Ruben, and I. L. Chaikoff, *J. Biol Chem.*, **122**, 169 (1937).

the labeled phosphatide content of organs at different intervals; some of their results are shown in Figures 51–55 (pages 275–277).

TABLE 105[a]

Ratio of Total and Specific Activities of Phosphatide Phosphorus of Rats on Olive Oil Diet and on Carbohydrate Diet[42]

Organ	Ratio of total activities	Ratio of specific activities
Intestine..	2	1.5
Liver...	1.6	1.2
Kidney...	1.3	1.1
Parenchymal organs, lungs, muscles, brain........	1	1

a Results shown were obtained four days after administering labeled phosphorus.

A marked effect of ingested oil on the formation of labeled phosphatides was also found to take place in stomach and large intestine, but the amount of active phosphatides formed per gram of stomach and large intestine was much smaller than that formed per gram of small intestine. This shows that the major part of the phosphatide turnover taking place in the digestive tract may be ascribed to the small intestine. The same applies to the digestive tract of the bird.[44] It was also found that removal of tissue very active in phosphatide formation (i.e., the gastrointestinal tract, and, to a minor extent, the kidneys) does not markedly influence new formation of phosphatides in the liver.

That the rate of renewal of phosphatides in liver is accelerated if the fat content of the circulation is increased was also shown in experiments on perfused cat liver.[45] With normal blood, 1.5% of liver phosphatide phosphorus was found to be replaced by active inorganic phosphorus added to the blood as sodium phosphate in the course of 2.5 hours, while with lipemic blood 2.7% was renewed. Thus, the effect of ingested fat on the rate of renewal of phosphatides is very pronounced in those organs which, like the intestinal mucosa and the liver, play a predominant part in fat metabolism.

Schmidt-Nielsen[46] found that, one hour after ^{32}P was administered by intramuscular injection to a rat, the specific activity of phospha-

[44] C. Entenman, S. Ruben, I. Perlman, F. W. Lorenz, and I. L. Chaikoff, J. Biol. Chem., 124, 795 (1938).

[45] L. Hahn and G. Hevesy, Biochem. J., 32, 342 (1938).

[46] K. Schmidt-Nielsen, Acta Physiol. Scand., 12, Suppl. XXXVII (1946).

tide phosphorus extracted from the intestine was four times larger, after feeding 2.5 g. peanut oil by stomach tube, than the specific activity of phosphatide phosphorus of the resting intestine. The increased ^{32}P content cannot be ascribed to the general increase in cell activity, because intestinal loops absorbing glucose did not synthesize phosphatides at a rapid rate but at the same low rate as nonabsorbing intestine. No increase was found in the total amount of phosphatides (about forty micromoles phosphatide phosphorus per gram intestine) present in the intestine during fat absorption. The newly formed phosphatide molecules must therefore either be transported away or split up again near the place of formation. Presumably the latter process takes place mainly. Poisoning with phlorizin does not decrease the rate of formation of phosphatides.

Fructose is phosphorylated at a rapid rate in the intestinal mucosa. Following intravenous injection of radioactive phosphorus, Kjerulf-Jensen observed a very rapid rate of renewal of fructose monophosphate in the intestinal mucosa of rabbits to which fructose had previously been fed. After a lapse of 4 minutes, all fructose phosphate present in the intestinal mucosa was found to be labeled. The amount of labeled fructose monophosphate was calculated to represent 60–100 mg. of fructose, rabbits being found under corresponding circumstances to absorb about 15 mg. fructose per minute. From these figures it was concluded that an intermediary phosphorylation, even of the total amount of fructose, can be maintained.[46a]

TABLE 106

Total ^{32}P Content and Specific Activity of Phosphatide P
in Small Intestine of Rats[47]

Diet	Time after ^{32}P administration, hr.	Per cent injected dose × 100 in isolated phosphatides[a]			
		Water	Choline	Oil + choline	Oil + water
Choline-deficient...	3	846 (38)	134 (48)	151 (47)	82 (38)
Choline-deficient...	6	132 (58)	143 (70)	267 (90)	133 (59)
Choline-deficient...	24	111 (42)	116 (48)	181 (61)	121 (48)
Stock............	6	117 (52)	145 (52)	233 (62)	187 (67)

[a] Figures in parentheses are the specific activity values. In these experiments rats weighing 100 g. were maintained for 7 days on a low-fat, low-choline diet. The ^{32}P was administered by intraperitoneal injection.

[46a] K. Kjerulf-Jensen, *Acta Physiol. Scand.*, **4**, 225 (1942).

[47] C. Artom and W. E. Cornatzer, *J. Biol. Chem.*, **165**, 393 (1946).

When single large doses of choline and fat are given simultaneously there is a considerable increase in both the total radioactivity and the specific activity of the intestinal phosphatides, as seen in Table 106. It is also shown that in rats on the choline-deficient diet, administration of oil alone does not affect the total radioactivity or specific activity values. This finding, in conjunction with results obtained from rats on a stock diet, suggests that the supply of choline (or choline precursors) may represent a limiting factor in the formation of phosphatides during the absorption of fat from the intestine.

D. RELATIVE SPEED OF FORMATION OF VARIOUS PHOSPHATIDES

Chargaff[48] compared the specific activity of the lecithin phosphorus and cephalin phosphorus extracted from 250 – 300-g. rats at 19 to 43 hours after oral administration of labeled sodium phosphate. The rate of turnover of cephalin was found to be somewhat higher as seen in Table 107.

TABLE 107

Relative Speed of Formation of Lecithin and Cephalin in Rats[48]

Rat No.	Time, hr.	Phosphatide	Weight, mg.	Relative speed of formation
1	19	Lecithin	1063.0	100
		Cephalin	353.0	113
2	43	Lecithin	935.3	140
		Cephalin	258.0	151

Comparison of specific activities of lecithin and cephalin phosphorus extracted 24 hours after oral administration of labeled sodium phosphate indicates that lecithin extracted from intestinal tract and liver is somewhat more active than the cephalin while the opposite behavior is shown by lecithin and cephalin of the brain. In normal liver, in experiments that lasted 24 hours, somewhat greater renewal figures were obtained for lecithin than for cephalin. Chargaff[48] (see also Chargaff et al.[49]) found the cephalin – lecithin ratio to be 0.8. Artom and colleagues[42] conducted experiments in which olive oil and labeled sodium phosphate were administered to rats nine hours before they were killed, and report the ratio to be about 0.6 (see also page 286).

[48] E. Chargaff, J. Biol. Chem., **128**, 587 (1939).
[49] E. Chargaff, K. B. Olson, and P. F. Partington, J. Biol. Chem., **134**, 505 (1940).

In rat carcinosarcoma 256, the specific activity of lecithin reaches a peak after 30 hours; cephalin attains its peak after 40 hours.[50] The rate of renewal of sphingomyelin in liver is slower than that of the other phosphatide fractions. In kidney about the same rate of renewal is found for all the phosphatide fractions.[51] The $^{32}\overset{.}{P}$ of sphingomyelin of rat organs, except in brain and muscles, reaches a maximum eight days after administration. At the end of eight days, the specific activity of liver declined to 0.027 and that of the intestinal mucosa to 0.010, while for skeletal muscle the specific activity is 0.3312, and for brain 0.3008 is found.[52,53]

Hahn and Tyrén[37] found a somewhat higher specific activity for lecithin phosphorus than for cephalin phosphorus extracted from rat and rabbit liver. In experiments taking 24 hours, brain cephalin was found to be more active than the brain lecithin.[49] After administration of labeled aminoethylphosphoric acid to rats, more newly formed lecithin than cephalin is found both in the liver and in the intestine. Aminoethylphosphoric acid was not found to be directly utilized in cephalin synthesis.[53a] In liver of the mouse during fasting, the specific activity figures for lecithin are uniformly higher than those for cephalin.[54]

E. EFFECT OF LIPOTROPIC SUBSTANCES ON PHOSPHATIDE TURNOVER IN LIVER

The striking effect of lecithin upon the liver of a depancreatized dog (maintained with insulin) was found to be due to its content of choline, which reduces the fat content of the liver of depancreatized rats as well as of rats fed a diet rich in fat. The fact that the rate of formation of new phosphatide molecules in the liver is accelerated by the administration of choline, was established by Perlman and Chaikoff.[55] Rats were fed for three days on a diet high in fat and low in protein; on the fourth day, half of each group was given 3 mg. of labeled phosphate and 30 mg. of choline chloride, simultaneously. The remaining half was given the labeled phosphate only. All animals were killed 4 hours after the

[50] F. L. Haven, *J. Natl. Cancer Inst.*, **1**, 205 (1940).
[51] F. E. Hunter and S. Levy, *J. Biol. Chem.*, **146**, 577 (1942).
[52] F. L. Haven and S. Levy, *J. Biol. Chem.*, **141**, 417 (1941).
[53] F. E. Hunter, *Proc. Soc. Exptl. Biol. Med.*, **46**, 287 (1941).
[53a] E. Chargaff and A. S. Keston, *J. Biol. Chem.*, **134**, 515 (1940).
[54] H. C. Hodge, P. L. MacLachlan, W. R. Bloor, E. A. Welch, S. Levy Kornberg, and M. Falkenheim, *J. Biol. Chem.*, **169**, 707 (1947).
[55] I. Perlman and I. L. Chaikoff, *J. Biol. Chem.*, **128**, 735 (1939).

administration of ^{32}P. While the phosphatides in the livers of the controls were found to contain 2.23% of the ^{32}P administered, the liver phosphatides of the choline-treated rats contained 2.92% (see also Patterson et al.[56]). Increased formation of labeled phosphatides was found to appear approximately 1 hour after choline ingestion, and its effect had disappeared about 10 to 12 hours later.

As distinct from choline, which promotes the formation of new phosphatide molecules in liver, cholesterol was found to have an opposite effect.

While the control diet consisted of 5 g. of unsalted butter to which was added 0.015 ml. of a standardized cod liver oil and 0.1 ml. of a vitamin B concentrate, the cholesterol diet contained the above constituents and added amounts of cholesterol varying from 250–400 mg. All rats were injected with labeled phosphate 26 hours after first receiving these diets, and their livers were removed 4 hours later. The liver phosphatides of the control rats contained, on the average, 2.9% of the ^{32}P administered, while the liver phosphatides of the rats fed cholesterol contained 2.3%.

In another set of experiments, choline and cholesterol were fed simultaneously. Rats were fed the cholesterol diet for 30 hours; they then received 30 mg. of choline chloride by stomach tube, and labeled phosphate subcutaneously. In these experiments, 5.07% of the ^{32}P administered was located in the liver phosphatides, while the corresponding figure for the control animals was only 2.81%.

Thus, combined feeding of choline and cholesterol clearly promotes the formation of new phosphatide molecules in the liver. All these cases are probably explained by an accelerated rate of renewal and not by an additional formation of phosphatide molecules.

Friedlander and colleagues[57] observed furthermore that the additional amounts of radiophosphatides formed under the influence of choline do not long remain in the liver. They pass into the plasma and increase the specific activity of plasma phosphatides. A single feeding of 300 mg. choline chloride per kg. body weight increases markedly the phosphatide turnover in the plasma, as seen in Figure 56. While after 12 hours the effect of choline is most pronounced, after the lapse of 96 hours the specific activity of the plasma phosphatides shows almost the same value as found in the controls.

The mechanism by which choline increases the specific activity of plasma phosphorus is not known. Since this occurs in the absence of a

[56] J. M. Patterson, N. B. Keevil, and E. W. McHenry, *J. Biol. Chem.*, **153**, 489 (1944).

[57] H. D. Friedlander, I. L. Chaikoff, and C. Entenman, *J. Biol. Chem.*, **158**, 231 (1945).

change in the total phosphatide content of the plasma, it would appear that choline increases utilization of phosphatides. Such an increase in utilization of phosphatides could mean either (1) an increased transport of phosphatides from liver to peripheral tissues or (2) an increased breakdown of choline-containing phosphatides within the liver.
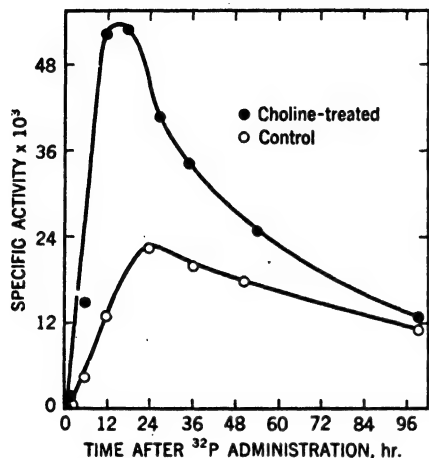
Fig. 56. Effect of choline on the specific activity–time relations of plasma phosphatide phosphorus in a dog.[57]

Patterson and associates found that choline deficiency resulted in decreased phosphatide turnover as well as in decreased concentration of phosphatides in liver[56] and kidneys[58] of rats. The fatty liver and hemorrhagic kidneys which result from choline deficiency are apparently due to this decrease in phosphatide turnover.

Patterson et al. observed that the rate of phosphatide turnover in rat kidney is greatest at the sixth day of life, when the kidney is most susceptible to choline deficiency, and that the turnover is greatly reduced in choline-deficient animals. This and other evidence is interpreted to indicate that the kidney lesion of choline deficiency can be ascribed to a failure of phosphatide synthesis.

Further evidence in support of the decreased rate of phosphatide turnover in choline deficiency is provided by studies[59] in which choline containing ^{15}N was fed to both normal and choline-deficient animals. Incorporation of new choline (tagged by the presence of ^{15}N) was found to be retarded in the choline-deficient rat.

The following results were obtained by Entenman et al.[60] in comparing the effect of choline on the specific activity of choline-containing and non-choline-containing phosphatide phosphorus. The specific activity–time curves, as seen in Figure 57, of choline-containing and non-choline-containing phosphatide phosphorus of liver are quite similar

[58] J. M. Patterson and E. W. McHenry, J. Biol. Chem., **156**, 265 (1944).

[59] E. G. Boxer and D. Stetten, Jr., J. Biol. Chem., **153**, 617 (1944).

[60] C. Entenman, I. L. Chaikoff, and H. D. Friedlander, J. Biol. Chem., **162**, 111 (1946).

in untreated dogs. A single ingestion of 300 mg. of choline per· kg. body weight greatly increases, however, the specific activities of choline-containing phosphatide phosphorus of liver. Ingested choline does
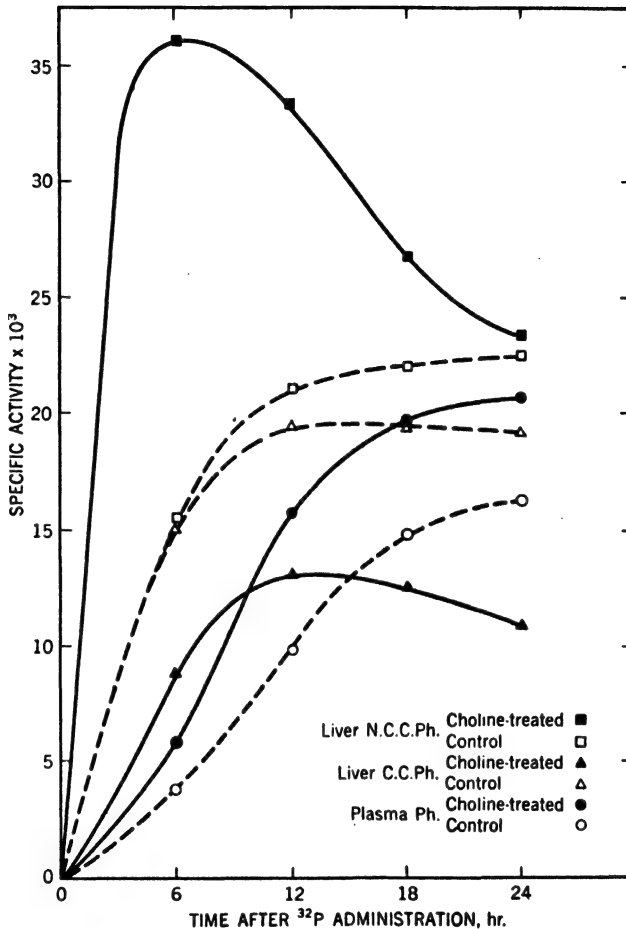
Fig. 57. Effect of choline on specific activity–time curve of choline-containing (C.C.Ph.) and non-choline-containing (N.C.C.Ph.) phosphatide phosphorus of liver.[60]

not increase specific activity of non-choline-containing phosphatide phosphorus of the liver. On the contrary, administered choline depresses its specific activity.

Specific activities of phosphatide phosphorus of various lobes of liver

were compared in the experiments described. The values were found to be uniform: for the left main lobe, the right middle lobe, the left middle lobe, and the right main lobe, the values 0.014, 0.015, 0.015, and 0.014, respectively, were obtained.

The effects of a single dose of methionine, cystine, and cysteine upon the phosphatide activity of the liver of rats fed a high-fat and low-protein diet were also investigated.[61] The amino acids were fed by stomach tube, simultaneously with injection of the labeled phosphate. The livers were analyzed 8 hours after the injection. An increase of about 30% was observed in the rate of renewal of liver phosphatides of rats given the amino acids. Amino acids differ in their capacity to stimulate phosphatide activity in liver. Glycine, alanine, serine, tyrosine, proline, glutamic acid, and asparagine were found to increase the rate of renewal of liver phosphatides. A negative result was obtained with taurine, creatine, dihydroxyethyl sulfoxide, and sarcosine.

Choline phosphate inhibits turnover of phosphatides in liver. The inhibition appears to be limited to the noncholine phosphatide fraction. Choline phosphate, as a unit, is probably not utilized in the synthesis of phosphatides.[62]

Ability of the organism to synthesize phosphatides is to a certain extent impaired by removal of a portion of the liver.

F. TURNOVER IN KIDNEY

The specific activity of phosphatides extracted from rabbit and rat kidney is lower than that of phosphatides from small intestine and liver at early intervals after administration of radioactive phosphate. After the lapse of six hours the same result was obtained in experiments with dogs, but after eighteen hours the specific activity of the kidney phosphatide remained lower than that of liver and about equal to that of small intestine. At 98 hours the specific activities of the phosphatides in all three tissue were roughly the same.[63] Acidosis induced by ingestion of ammonium chloride increases turnover of kidney phosphatides, according to Weissberger.[64] Administration of phlorizin did not influence turnover rate of phosphatides in kidneys of the rat.[65]

[61] I. Perlman, N. Stillman, and I. L. Chaikoff, *J. Biol. Chem.*, **133**, 651 (1940); **135**, 359 (1940).

[62] R. F. Riley, *J. Biol. Chem.*, **153**, 535 (1944); *J. Am. Chem. Soc.*, **66**, 512 (1944).

[63] I. L. Chaikoff, *Physiol. Revs.*, **22**, 291 (1942).

[64] L. H. Weissberger, *J. Biol. Chem.*, **132**, 219 (1940).

[65] L. H. Weissberger, *J. Biol. Chem.*, **139**, 543 (1941).

G. TURNOVER IN MUSCLES

[32]P slowly appears as phosphatide phosphorus in skeletal muscle. This may be related to the slow rate of formation or entry of the labeled phosphatides into the muscle cells. The specific activity of phosphatides extracted from cardiac muscle, into which the phosphate penetrates at a higher rate than into skeletal muscle, is found to be higher.[66] Artom[67] (see also Friedlander et al.[68]) injected radioactive phosphate into rats and cats in which the femoral and sciatic nerves of one leg had previously been cut. In the denervated muscle, newly labeled phosphatides and, to a smaller extent, total phosphatides were found to be increased. The specific activity values of phosphatide phosphorus showed a gradient in the following order: liver > plasma > denervated muscle > intact muscle. The same gradient for the specific activities of the phosphatides was obtained in an experiment after introduction of a labeled emulsion of radioactive phosphatides. It is doubtful if these results can be explained by assuming that the labeled phosphatides synthesized by liver (see page 304) and released into the plasma penetrate the muscle cells, larger amounts being deposited in the denervated muscles. Hevesy and Hahn,[2] four hours after injecting plasma which contained labeled phosphatides into the circulation of the rabbit, found 2.5% of the labeled phosphatides to be present in the muscles only and, in the experiments of Zilversmit et al.[68a] on dogs, after a lapse of five hours the corresponding figure was found to be 4.5.

The radioactive phosphorus taken up by the muscles of adult rats (weighing 250–300 g.) as phosphatide phosphorus 48 hours after subcutaneous injection is seen in Table 108; in Table 109 the percentage uptake of the total ^{32}P is given. 5.3% of the ^{32}P content of the muscles is thus present as phosphatide phosphorus in the muscles 48 hours after the start of the experiment.

Denervation was also observed by Friedlander and associates[68] to be followed by a pronounced increase in capacity of the muscle to deposit labeled phosphatides. This change made its appearance before appreciable atrophy of the muscle set in and was still observed 19 days

[66] C. Entenman, S. Ruben, I. Perlman, F. W. Lorenz, and I. L. Chaikoff, J. Biol. Chem., 124, 795 (1938).

[67] C. Artom, J. Biol. Chem., 139, 953 (1941).

[68] H. D. Friedlander, I. Perlman, and I. L. Chaikoff, Am. J. Physiol., 132, 24 (1941).

[68a] D. B. Zilversmit, C. Entenman, M. C. Fishler, and I. L. Chaikoff, J. Gen. Physiol., 26, 333 (1943).

after section of the nerve. The uptake of total ^{32}P by the muscles during the first 48 hours of the experiment was found to be only with few per cent larger than the uptake during the first 12 hours. This result was to be expected in view of the decline of the ^{32}P level of the blood plasma in the course of the experiment. The percentage labeled phosphatide formation is, however, appreciably augmented with increasing time, 0.0024 and 0.0064% of the labeled phosphorus administered being found as phosphatide phosphorus per gram muscle after the lapse of 12 and 48 hours, respectively.

TABLE 108

^{32}P Present as Phosphatide Phosphorus in Rat Muscles
48 Hours after Administration[68]

Muscle wt., g.[a]	^{32}P in phosphatides, per cent administered per g. muscle $\times 10^2$
0.95, 0.91	0.71, 0.96
1.13, 1.11	0.58, 0.66
1.35, 1.36	0.64, 0.79
1.54, 1.44	0.42, 0.60

[a] The first value on each line is for the right muscle; the second, for the left.

TABLE 109

^{32}P in Rat Muscle 48 Hours after Administration[68]

Muscle wt., g.[a]	^{32}P in muscle, per cent administered per g. muscle
1.38, 1.39	0.14, 0.12
1.39, 1.40	0.14, 0.14
1.52, 1.55	0.14, 0.13
1.73, 1.75	0.10, 0.10

[a] The first value on each line is for the right muscle; the second, for the left.

The turnover rate of phosphatides is found to be increased in muscle of rats maintained on a diet deficient in fat.[69] In the fasting mouse, all phosphatide fractions, with the exception of α-cephalin, which remained constant, showed a large increase in rate of renewal and reached a maximum on the second day of fasting.[70]

[69] G. Hevesy and I. Smedley-Maclean, *Biochem. J.*, **34,** 903 (1940).
[70] H. C. Hodge, P. L. MacLachlan, W. R. Bloor, E. A. Welch, and S. Levy, *Federation Proc.*, **2,** 63 (1943).

H. TURNOVER IN BRAIN

Brain is the organ in which both rate of penetration of labeled phosphate and incorporation of ^{32}P into phosphatides is found to be lower than in any other organ; see Table 110.

TABLE 110
Formation of Labeled Phosphatides in Rabbit Brain[71]

Duration of experiment	Ratio of specific activities of			
	brain inorganic P to plasma inorganic P	brain phosphatide P to plasma inorganic P	brain phosphatide P to brain inorganic P	brain phosphatide P to liver phosphatide P
250 min..........	0.015	0.0002	0.016	0.005
11.5 hr..........	0.030	0.0033	0.11	0.022
9 days..........	0.19	0.054	0.29	0.63
50 days..........	0.56	0.43	0.77	0.43

If and to what extent labeled phosphatides migrate from plasma into brain cells is not known. That labeled phosphatide molecules can be built up in brain tissue follows from experiments carried out with brain tissue slices, described on page 297.

The maximum ^{32}P content (0.06% of the labeled phosphate administered per g. tissue) was observed in adult rat brain 200 hours after administration. In young brain, maximum content was observed only after 300 hours.[73]

As the tissue formed in an organism given a labeled phosphate will necessarily become labeled, and, furthermore, as there is usually in the growing organism intensified enzyme action which leads to an accelerated rate of renewal of tissue compounds, we should expect a rapid new formation of phosphatides to take place in brains of growing rats. Rats of very different ages (including newly born rats) were studied by Changus et al.[72] and Fries et al.[73] The highest rate of formation of labeled phosphatides was found to take place at birth. Though this general characteristic was shared by all the parts investigated the phosphatide activity was by no means uniform throughout the nervous system; striking differences were encountered in the formation of

[71] G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **15**, 5 (1940).

[72] G. W. Changus, I. L. Chaikoff, and S. Ruben, *J. Biol. Chem.*, **126**, 493 (1938).

[73] B. A. Fries, G. W. Changus, and I. L. Chaikoff, *J. Biol. Chem.*, **132**, 23 (1940).

labeled phosphatides in forebrain, cerebellum, medulla, and spinal cord, as seen in Figure 58.
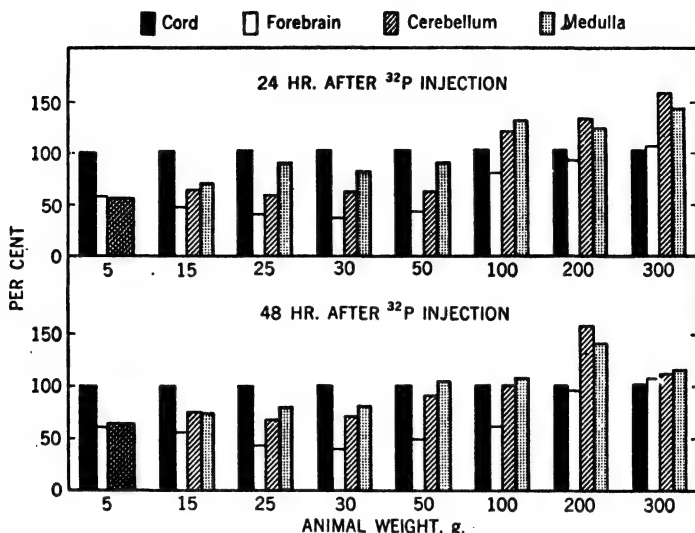
Fig. 58. Comparative phosphatide activities of forebrain, cerebellum, medulla, and spinal cord from birth until the time rat attains a weight of 300 g.[73] In each age group, the spinal cord has the arbitrary value of 100. Cross-hatched areas for the 5-g. rat represent combined values of medulla and cerebellum.

From birth until the time the rat attains a weight of 50 g., a precipitous decline in active phosphatide content occurs throughout the central nervous system of a rat born from parents to which ^{32}P was administered previous to the birth of the rat. So striking is this loss in activity of brain phosphatides that, by the time the rat reaches a weight of 50 g., the spinal cord retains only 5% of the activity present in the newly born rat. During this period, the specific activity of plasma phosphate declines sharply, owing to a "dilution" of the ^{32}P atoms of the plasma by nonactive phosphorus taken up with the food or given off by the organs. Active phosphatide molecules of the brain become less active by being degraded and subsequently resynthesized with incorporation of less active inorganic phosphate. Thus, a considerable reduction in activity of brain phosphatides will indicate a high rate of renewal of phosphatide molecules of the central nervous system.

An abrupt change in rate of renewal of brain phosphatides occurs in

the central nervous system of the rat during its growth from 30 to 50 g. As growth proceeds beyond 50 g., activity of brain phosphatides decreases throughout the central nervous system, but at a much lower rate than observed between birth and the age when the weight of 50 g. is attained. The spinal cord in the 200-g. rat possesses an activity of 20% of that of the 50-g. rat, whereas in the 300-g. rat, the cord retains 15% of the activity found in the 50-g. animal. Forebrain, cerebellum, and medulla also lose activity as the animal grows from 50 to 300 g., but the rate of decline in activity is lower than that occurring in spinal cord (see Fig. 59). By the time a weight of 200 or 300 g. is reached, the relative activities of phosphatides of the forebrain, cerebellum, and medulla are as great as those of spinal cord, or even greater.

Fig. 59. Comparison of decline in phosphatide activity of forebrain, cerebellum, medulla, and spinal cord during the time the rat weighs from 50 to 300 g.[73] Symbols represent activities 24 hours after administration of labeled P.

I. ADRENAL GLANDS AND PHOSPHATIDE FORMATION

Ability of the adrenalectomized animal to synthesize new phosphatide molecules was established. The rate of formation of labeled phosphatide molecules in liver and small intestine of the rat is not influenced by complete removal of both adrenal glands,[35c] nor is the rate of incorporation of deuterium-containing fatty acids into the phosphatide molecules affected.[74]

J. TURNOVER IN NEOPLASTIC TISSUE

If the phosphatide molecules of carcinomatous tissue were replaced at a high rate by labeled molecules, and such molecules were given off by the tumor to the circulation shortly after the administration of labeled phosphate, the presence of carcinomatous tissue could possibly be diagnosed by determination of the activity of plasma phosphatides.

[74] R. H. Barnes, E. S. Miller, and G. O. Burr, *J. Biol. Chem.*, **140**, 241, 247 (1941).

The facts, however, that phosphatide turnover of most neoplastic tissue is much slower than turnover in liver and intestinal mucosa and that phosphatide molecules in the circulation were to a very large extent built up in the liver frustrate this possibility.

No appreciable difference is found in labeled phosphatide formation of spontaneous and transplanted tumors.[75] In both cases, it is appreciably larger than that occurring in muscles, but smaller than that found in liver. Thus, the formation of active phosphatide molecules in carcinomatous tissue is neither extremely pronounced nor very low.

A detailed investigation of phosphatide metabolism in mammary carcinoma, lymphoma, lymphosarcoma, and sarcoma 180 was carried out by Jones, Chaikoff, and Lawrence.[76-77a] Four tumors were transplanted into mice. They differed with respect to cell type and rate of growth. Two of the tumors produced metastases in distant parts, whereas the two others remained entirely localized at the place of inoculation. Uniform phosphatide activity was not found in the several types of tumor examined. The activity of lymphoma was only about one-third of that found in mammary carcinoma or lymphosarcoma. Cell type is apparently not a decisive factor in determining extent of phosphatide activity. Each tumor possesses a characteristic phosphatide turnover, which is independent of the host.

The maximum deposition of phosphatide ^{32}P in neoplastic tissues may last from 10 to 50 hours.

In carcinosarcoma 256 the turnover of lecithin is somewhat more rapid than that of cephalin. The mode of behavior of the sphingomyelin fraction was found to be similar to that of cephalin but unlike that of the lecithin fraction of the same tumor.[78] The specific activity of sphingomyelin phosphorus increases to a maximum at 48 hours after feeding labeled phosphate. Cephalin shows the same behavior in contrast to lecithin.[79]

K. TURNOVER IN BLOOD

Shortly after administration of labeled phosphate, tagged phosphatides penetrate from the liver into the circulation. By 40 hours one-

[75] G. Hevesy, *Acta Unio Intern. contra Cancrum*, **4**, 175 (1939).

[76] H. B. Jones, I. L. Chaikoff, and J. H. Lawrence, *J. Biol. Chem.*, **133**, 319 (1940).

[77] H. B. Jones, I. L. Chaikoff, and J. H. Lawrence, *J. Biol. Chem.*, **128**, 631 (1939).

[77a] H. B. Jones, T. L. Chaikoff, and J. H. Lawrence, *Am. J. Cancer*, **40**, 235 (1940).

[78] F. L. Haven, *J. Natl. Cancer Inst.*, **1**, 205 (1940).

[79] F. E. Hunter and S. Levy, *J. Biol. Chem.*, **146**, 577 (1942).

tenth to five-tenths per cent of the administered ^{32}P has been incorporated into phosphatides in the total plasma of the dog.[81] In experiments in which labeled inorganic phosphorus in rabbit plasma was kept at a constant level,[82] the phosphatide phosphorus of the plasma showed

TABLE 111

Specific Activity of Inorganic and Phosphatide Phosphorus of Rabbit Plasma[80]

Time	Relative specific activity		Time	Relative specific activity	
	Inorganic P	Phosphatide P		Inorganic P	Phosphatide P
4 hours	100	0.53	45 hours	100	22.0
16 hours	100	3.8	55 hours	100	27.5
25 hours	100	8.1	9 days	100	81.6
37 hours	100	15.0			

the specific activities recorded in Table 111. To what extent labeled phosphatide molecules are·built up in the plasma can be investigated only by experiments *in vitro*. In such an experiment (66), lasting 4.5 hours, the specific activity of the phosphatide phosphorus was found to be less than 0.1% that of the inorganic phosphorus.[82]

L. PHOSPHATIDE FORMATION IN TISSUE SLICES

The question whether a tissue can synthesize phosphatides independently or whether it acquires the phosphatides from the plasma only after their formation by a more active tissue, was answered by Fries *et al.*[83] in the following manner. A sciatic nerve of a dog stripped free of all adipose and connective tissue and weighing 300 mg. was placed in 5 ml. of Ringer solution containing radioactive phosphate. For control purposes the adipose–connective tissue surrounding the nerve was treated in a similar way. Conversion of radiophosphate from the Ringer solution into radiophosphatide by the nerve was found to be considerable, as is seen in Table 112. These experiments show that the nerve process, separated from the nerve cell body, can form phosphatides from inorganic phosphate.

[80] G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **15, 5** (1940).

[81] M. C. Fishler, C. Entenman, M. L. Montgomery, and I. L. Chaikoff, *J. Biol. Chem.*, **150**, 47 (1943).

[82] L. Hahn and G. Hevesy, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, **22**, 188 (1938).

[83] B. A. Fries, H. Schachner, and I. L. Chaikoff, *J. Biol. Chem.*, **144**, 59 (1942).

Similar values were obtained for formation of radioactive phosphatide in brain slices of young and old rats. In the course of 4 hours 0.70 to 0.85% of the labeled phosphorus of Ringer solution was incorporated in

TABLE 112

Formation of Radioactive Phosphatide[a] by Dog Sciatic Nerves[83]

Time interval, hr.	Nerve	Adipose–connective tissue
0	0.006	0.008
0	0	0
4	0.63	0.036
4	0.72	0.026
4	0.44	——

[a] All values are expressed as per cent labeled phosphorus of the Ringer solution incorporated into phosphatide per gram wet tissue.

TABLE 113

Formation of Radioactive Phosphatides[a] by Brain[83]

Time interval, hr.	Brain slices						Brain homogenate					
	15-g. rat		50-g. rat		200-g. rat		15-g. rat		50-g. rat		200-g. rat	
	Wet	Dry[b]	Wet	Dry[b]	Wet	Dry[b]	Wet	Dry	Wet	Dry	Wet	Dry
0	0.0067	0.054	0.0058	0.028	0.0093	0.043	0.0047	0.038	0.023	0.11	0.027	0.13
0	0.0047	0.038	0.014	0.067	0.013	0.061	0	0	0	0	0.015	0.070
0	0.0067	0.054	0.0035	0.017	0.0096	0.045	0.0093	0.075	0	0	0.035	0.16
Av.	*0.0060*	*0.049*	*0.0078*	*0.037*	*0.011*	*0.050*	*0.00500*	*0.038*	*0.007*	*0.04*	*0.026*	*0.12*
1	0.32	2.6	0.29	1.4	0.15	0.70	0.13	1.0	0.064	0.27	0.075	0.24
1	0.33	2.7	0.32	1.6			0.11	0.91	0.049	0.20	0.074	0.24
1	0.34	2.7	0.25	1.2	0.21	0.96	0.10	0.89	0.056	0.23	0.086	0.29
2	0.53	4.3	0.38	1.8	0.42	2.0	0.15	1.2	0.079	0.38	0.075	0.24
2	0.64	5.1	0.51	2.4	0.51	2.4	0.16	1.3	0.074	0.36	0.088	0.30
2	0.65	5.3	0.47	2.1			0.16	1.3	0.062	0.26	0.078	0.26
4	0.70	5.7	0.52	2.5	0.47	2.2	0.22	1.8	0.097	0.46	0.12	0.57
4	0.84	6.8	0.46	2.2	0.57	2.7	0.21	1.7	0.095	0.45	0.095	0.45
4	0.85	6.9	0.55	2.6	0.52	2.4	0.20	1.6	0.082	0.36	0.083	0.39

[a] All values are expressed as per cent of labeled phosphorus of the bath incorporated into phosphatide per gram of tissue. All values recorded for 1, 2, and 4 hours have had the average zero-time value subtracted.

[b] The water content of the forebrain of 15-, 50-, and 200-g. rats is 87.6, 79.2, and 78.6%, respectively.

brain slices (per gram wet tissue); in brain homogenate lower values (0.20 to 0.22) were obtained, as is seen in Table 113.

This type of phosphorylation can only be detected by making use of labeled phosphate. The usual methods of chemical analysis fail to detect the synthesis of the small percentage of new phosphatide molecules, as the formation of these goes hand in hand with the autolysis of a comparatively large percentage of phosphatide molecules present at the start of the experiment. The amount of phosphatide found in brain homogenate after 4 hours is 10 – 15% less than that present at zero time.

Conversion of labeled, inorganic phosphorus into phosphatide phosphorus by surviving brain slices is greatly increased (up to about five times) by the addition of hexose, glucose, galactose, mannose, and fructose to the bicarbonate – Ringer solution containing labeled phosphate, as is seen in Figure 60. This increase in formation of labeled phosphatide could be due either to an increased rate of penetration of labeled phosphate into the site of synthesis of phosphatides, or to an enhanced rate of formation of labeled phosphatide molecules. That the presence of hexoses accelerates the rate of formation of labeled phosphatide molecules is shown by the following experiment. Brain slices, after being kept in a bicarbonate – Ringer solution containing ^{32}P, are washed and placed in an inactive

Fig. 60. Effect of various concentrations of glucose on recovery of radiophosphatide in brain slices at various time intervals.[84]

bicarbonate – Ringer solution for two hours. While in the glucose-free Ringer solution hardly any further formation of labeled phosphatides takes place, a threefold increase of labeled phosphatide content is observed when the Ringer solution contains glucose.

It is well known that the oxygen consumption of brain preparations remains nearly constant for long periods when the preparations are placed in a Ringer medium containing glucose, whereas in a glucose-free medium the oxygen uptake decreases rapidly. Presumably the forma-

[84] H. Schachner, B. A. Fries, and I. L. Chaikoff, *J. Biol. Chem.*, **146**, 95 (1942).

tion of labeled phosphatide molecules is promoted by increased oxygen consumption which provides increased oxidative energy for formation of phosphatide or of a phosphorus-containing phosphatide precursor. The stimulatory effect of the hexose upon formation of radiophosphatide does not occur under anaerobic conditions. The stimulation is abolished when the tissue organization is disrupted by homogenization. Addition of pentoses fails to increase the yield of radiophosphatides.[84]

TABLE 114

Effect of Various Oxygen Tensions on Phosphatide Formation
in Liver Slices[86]

Gas mixture in atmosphere above slices[a]			Per cent added ^{32}P recovered as phosphatide per gram wet wt.		
O_2, %	N_2, %	CO_2, %	Rat 1	Rat 2	Rat 3
0	95	5	0.18	0.15	0.79
5	90	5	1.6	1.7	2.7
10	85	5	2.5	2.3	2.9
21	79		3.3	2.9	4.0

[a] Period of incubation = 2 hr.

In both liver and kidney slices, phosphatide formation is greatly impaired in the absence of oxygen, as is seen in Table 114. Phosphatide formation in surviving liver and kidney slices is extremely sensitive to the presence of sodium cyanide. Concentrations as low as 0.001 M sodium cyanide inhibit the formation in liver slices to the extent of about 90%, as is seen in Table 115.

Azide and hydrogen sulfide were found to have an effect similar to that shown by cyanide on formation of labeled phosphatides in tissue slices. Carbon monoxide was furthermore shown to have an inhibitory effect, which is more pronounced in the dark than in the presence of strong light (see Table 116).

Homogenized liver tissue completely loses its ability to incorporate phosphate into the phosphatide molecule.[85] Much less phosphatide formation is shown by homogenates of kidney and brain (see Table 113, p. 298) than by slices of these tissues.[87] The loss or decrease in activity of disintegrated tissue may be due to dilution of substances essential for

[85] M. C. Fishler, A. Taurog, I. Perlman, and I. L. Chaikoff, *J. Biol. Chem.*, **141**, 809 (1941).

[86] A. Taurog, I. L. Chaikoff, and I. Perlman, *J. Biol. Chem.*, **145**, 281 (1942).

TABLE 115

Effect of Cyanide on Phosphatide Formation during Two-Hour Incubation[88]

Rat No.	Cyanide, concentration, M	Per cent added ^{32}P recovered as phosphatide per g. tissue				Inhibition, %
		Control, cyanide absent		Cyanide present		
		Wet weight	Dry weight	Wet weight	Dry weight	
Liver						
1	0.03	1.6	7.0	0.065	0.29	96
2	0.03	2.4	11	0.011	0.048	100
3	0.01	1.7	7.5	0.098	0.43	94
4	0.01	2.2	9.7	0.015	0.066	99
5	0.01	2.2	9.7	0.057	0.25	97
6	0.01	2.2	9.7	0.29	1.3	87
7	0.01	3.0	13	0.14	0.62	95
8	0.005			0.054	0.24	
9	0.003	2.1	9.2	0.24	1.1	89
10	0.003	2.9	13	0.25	1.1	92
11	0.001	3.2	14	0.32	1.4	90
11	0.001	3.2	14	0.39	1.7	88
Kidney						
12	0.01	2.3	13	0.00	0.00	100
13	0.01	1.9	11	0.00	0.00	100
14	0.005	1.8	10	0.00	0.00	100
15	0.005	1.5	8.4	0.00	0.00	100

TABLE 116

Effect of Light on Carbon Monoxide Inhibition of Phosphatide Formation in Liver Slices Incubating Two Hours[88]

Rat No.	Per cent added ^{32}P recovered as phosphatide per g. tissue						Inhibition, %	
	Control		CO in light		CO in dark			
	Wet weight	Dry weight	Wet weight	Dry weight	Wet weight	Dry weight	CO in light	CO in dark
1	1.9	8.4	1.4	6.2	1.1	4.8	26	42
2	1.5	6.6	0.87	3.8	0.59	2.6	42	61
3	2.1	9.2	1.1	4.8	0.68	3.0	48	68

[87] B. A. Fries, H. Schachner, and I. L. Chaikoff, *J. Biol. Chem.*, **144**, 59 (1942).
[88] A. Taurog, I. L. Chaikoff, and I. Perlman, *J. Biol. Chem.*, **145**, 281 (1942).

the synthesis or to their destruction by enzymes.[89] In surviving rat kidney and liver slices much more radioactive organic phosphorus is found when the uptake of ^{32}P is investigated in the presence of fluoride.[90]

One may be tempted to interpret the formation of labeled phosphatides in tissue slices as due to partial reversibility of hydrolysis of phosphatides.   Even though the tendency of the reaction is far in the direction of decomposition, the reverse reaction may occur to a slight degree even during the early period of forward reaction.   The results obtained by Chaikoff and associates with respiratory inhibitors exclude, however, the possibility that the formation of labeled phosphatides is due to a reversal of the decomposition of phosphatides proceeding in the tissue slices.

M. PATH OF CONVERSION OF INORGANIC PHOSPHATE TO PHOSPHATIDE

The path of conversion of inorganic phosphate to phosphatide is not known.   Glycerophosphate, diglycerides, neutral fat, choline phosphate, phosphoproteins, or other compounds may be involved as intermediates. That aminoethylphosphoric acid can be excluded from this group of substances follows from the work of Chargaff and Keston.[91]   Experiments in which 80 mg. of labeled disodium aminoethyl phosphate was administered to adult rats by subcutaneous injection showed that the body was unable to utilize aminoethylphosphoric acid as such for the synthesis of cephalin.   Of the ^{32}P administered as aminoethylphosphoric acid, 28% was found to be excreted through the kidneys in the course of eight hours in these experiments.   (Compare also the work of Chargaff, Olson, and Partington.[91a])

Enzymic hydrolysis of aminoethylphosphoric acid in the tissues is presumably followed by utilization of the inorganic phosphate for synthesis of lecithin and of demethylation of lecithin to form cephalin. The aminoethylphosphoric acid normally occurring in the body tissue may be a product of catabolism of cephalin.

Glycerophosphate and phosphorylethanolamine containing ^{32}P were prepared by Chaikoff et al.,[89] and their incorporation into phosphatides of liver and kidney was demonstrated by surviving slices and also in the

[89] I. L. Chaikoff, *Physiol. Revs.*, **22**, 291 (1942).

[90] N. O. Kaplan, I. Memelsdorf, and E. Dodge, *J. Biol. Chem.*, **160**, 631 (1945).

[91] E. Chargaff and A. S. Keston, *J. Biol. Chem.*, **134**, 515 (1940).

[91a] E. Chargaff, K. B. Olson, and P. F. Partington, *J. Biol. Chem.*, **134**, 555 (1940).

intact animal. These experiments gave no proof that breakdown of these labeled compounds to inorganic phosphate did not occur before conversion of the radioactive phosphorus to phosphatide.

N. RATE OF INTERCHANGE OF PLASMA PHOSPHATIDES WITH TISSUE PHOSPHATIDES

Rate of interchange of plasma phosphatides with tissue phosphatides was determined in experiments in which part of the plasma of a rabbit was replaced by an equal volume of plasma from another rabbit con-
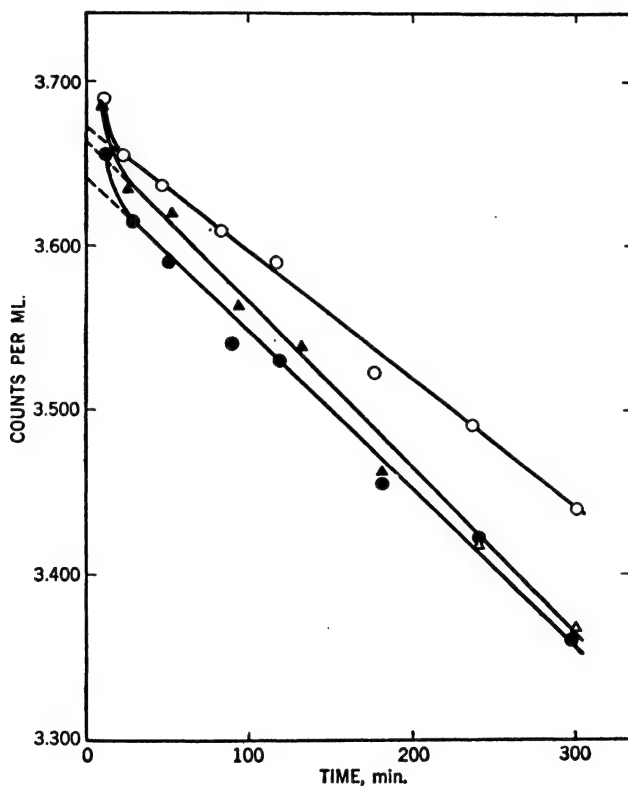
Fig. 61.   Rate of disappearance of labeled phosphatides from plasma of three different dogs.[93]

taining labeled phosphatides[92]; similar experiments were also carried out with chicks[92] and with dogs.[93] In another investigation an emulsion of

[92] G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **15,** No. 6 (1940).

[93] D. B. Zilversmit, C. Entenman, M. C. Fishler, and I. L. Chaikoff, *J. Gen. Physiol.*, **26,** 333 (1943).

phosphatides prepared from rat's liver was introduced into the circulation of a rat.[94] In the course of 10 hours a substantial part of the labeled phosphatide molecules were found to have left the plasma and were detected in different organs, especially in the liver. Some results obtained in experiments with dogs[93] are seen in Table 117 and in Figure 61.

TABLE 117

Distribution of Phosphatides in Tissues of the Dog at End of Five Hours[93]

Tissue	Injected dose of labeled phosphatides per whole organ, %	Labeled phosphatide P per whole organ, mg.	Organ phosphatides supplied by plasma phosphatides per hr., %
Plasma...................	53.0	18.5[a]	—
Liver	11.	173	1.09
Kidney...................	1.13	28.6	0.67
Small intestine...........	2.44	68	0.61
Spleen...................	0.35	8.5	0.71
Red corpuscles...........	1.15	——	—
Muscle...................	4.2	——	—

[a] Milligrams phosphatide P per 100 milliliters.

Labeled phosphatide found in liver and other organs originated almost exclusively from the plasma, though the plasma injected contained, beside labeled phosphatide, an appreciable amount of free labeled phosphate as well. Of the 2,540,000 radioactive units, 600,000 were present in compounds other than phosphatides. (Three millicuries active phosphate was injected into the dog 26 hours before the plasma transfusion.) The amount of labeled phosphatides formed from free phosphate after transfusion of labeled plasma can almost be disregarded, as in 5 hours only 0.01% of the injected organic ^{32}P was converted to phosphatide phosphorus per gram liver. Of the 14,400 counts of phosphatide phosphorus found per gram liver, only about 60 counts were thus synthesized in the liver.

During the postabsorptive phase 5.2 to 8.0 mg. phosphatide phosphorus is turned over per hour in the plasma of dogs weighing 6 – 9 kg. In experiments with rabbits, after the lapse of 4 hours, 29 – 38% of the labeled phosphatide molecules originally present in the plasma were found in the liver.[92] In the course of 17 minutes, 63% of labeled phos-

[94] F. L. Haven and W. F. Bale, *J. Biol. Chem.*, **129**, 23 (1939).

phatides originally present in the plasma of the chick left the circulation.[92]

It is in the liver that the phosphatide interchange takes place at the highest rate. The liver is thus not only the main organ in which phosphatide molecules are built up (see also page 275), but also that showing the greatest permeability to phosphatide molecules.

The fact that 76–83% of the injected labeled phosphatides can be accounted for in the seven tissues examined suggests that the breakdown of phosphatides in the animal tissue cannot be a rapid process.[93]

That beside being the principle tissue in the body concerned with the synthesis and supply of plasma phosphatides the liver is also mainly responsible for the removal of phosphatides is shown by further work of Entenman and associates.[95] When comparing the rates at which the injected labeled plasma phosphatides disappeared from the plasma of the normal and the liverless dogs, the rate of disappearance from the plasma of the normal dog was found to be six to ten times larger than the rate of disappearance from the liverless dog. The specific activities of the plasma phosphatide phosphorus of dogs that have received intravenously radioactive plasma phosphatides did not decrease significantly after the liver had been excluded from the circulation. In contrast to this result the labeled phosphatides disappeared at a normal rate from the plasma when the gastrointestinal tract was removed. While in normal dogs weighing from 7 to 18 kg. the plasma phosphatides are completely turned over in 6 to 10 hours, by depriving these dogs of their livers the time required for complete turnover was prolonged to 33 to 160 hours.

O. PASSAGE OF PHOSPHATIDES INTO LYMPHATIC CHANNELS

As mentioned above, in the course of few hours a very appreciable part of the phosphatide molecules originally present in the plasma are found to be replaced mainly by phosphatide molecules previously present in the liver. Reinhardt et al.[96] found that part of the labeled phosphatide molecules injected into plasma reached lymphatic channels and were recovered in the thoracic duct lymph. It is not established through which tissues phosphatides migrate, although liver presumably

[95] C. Entenman, I. L. Chaikoff, and D. B. Zilversmit, *J. Biol. Chem.*, **166**, 15 (1946).

[96] W. O. Reinhardt, M. C. Fishler, and I. L. Chaikoff, *J. Biol. Chem.*, **152**, 79 (1944).

plays an important part in this process. Some figures for the recovery of injected labeled plasma phosphatides are seen in Table 118.

TABLE 118

Recovery of Injected Plasma Radiophosphatide in Thoracic Duct Lymph of a Dog[96]

	Lymph				Plasma
Interval after injection of plasma, min.	Total lymph collected, ml.	Rate of flow, ml./min.	Phosphatide ^{32}P expressed as radioactive counts/min.		Phosphatide ^{32}P expressed as radioactive counts/ min./ml.
			Per ml.	For total sample collected	
Before	9.9	0.49			
4 before to 40 after	23.9	0.54	94	2250	——
40–90	26.4	0.53	216	5680	——
90–153	23.3	0.37	251	5850	937
153–214	23.9	0.39	213	5100	806
214–244	13.4	0.45	191	2560	——
244–272	13.3	0.47	191	2540	——
272–319	13.2	0.28	167	2220	——
319–359	13.6	0.34	178	2420	504

The dog (weight, 17.9 kg.) received intravenously 100 ml. of plasma containing a total of 759,200 counts per minute as phosphatide. The first sample of lymph was obtained during the first 36 minutes after the injection of radiophosphatide. This sample already contained over 2000 counts per minute. If we assume that plasma represents 5% of the body weight, the plasma still contained a total of 452,000 counts per minute at the end of this period of observation. Approximately 9% of the phosphatides that left the plasma was recovered from the lymph of the thoracic duct in the course of 6 hours and still higher figures (20%) were obtained in experiments with other dogs. Thus thoracic duct lymph serves as a medium for the return to the plasma of a significant fraction of the phosphatides lost from the plasma.

P. ORIGIN OF PLASMA PHOSPHATIDES

Strong additional evidence that plasma phosphatides are mainly derived from liver is provided in investigations on formation of labeled phosphatides in the hepatectomized dog by Fishler et al.[97] The observation that radiophosphatides are found in the kidney and small intestine of the hepatectomized dog leaves no doubt that the liver is not the only

[97] M. C. Fishler, C. Entenman, M. L. Montgomery, and I. L. Chaikoff, *J. Biol. Chem.*, **150**, 47 (1943).

site of phosphatide formation in the animal body. The recoveries of phosphatide ^{32}P per gram kidney phosphatide or per gram small intestine phosphatide in the liverless dog do not differ significantly from those found in the intact dog, the specific activities of kidney and intestinal mucosa phosphorus being, after the lapse of five hours, 65 and 41%, respectively, of that of liver phosphatide phosphorus. Nevertheless, only negligible amounts of phosphatide ^{32}P were recovered from the plasma of the hepatectomized dog as late as 6 hours after excision of the liver, as is seen in Table 119. For sake of comparison the figures

TABLE 119

Recovery of Intravenously Injected ^{32}P as Phosphatide ^{32}P in Plasma and Tissues of Hepatectomized Dog[97]

Dog weight, kg.	Time killed after ^{32}P injection, hr.	Phosphatide content at end of experiment, mg./100 g. tissue			Recovery of phosphatide ^{32}P as per cent of injected [32]P			
					Plasma		Kidney	Small Intestine
		Plasma	Kidney	Small intestine	Per ml. $\times 10^6$	Per g. phosphatide $\times 10^3$	Per g. phosphatide	Per g. phosphatide
Hepatectomy								
18.0	2	270	2380		0.1	0.03	0.25	
17.7	3	163	2030	1140	4	3	0.27	0.091
20.2	4	300			15	5		
20.0	4.5	210			19	9		
22.0	4.5	344	1820	1180	5	2	0.23	0.12
22.2	6	153	1470	1960	3	2	0.18	0.032
19.8	6	204	1870	1100	6	3	0.40	0.10
20.0	6	315	1860	2070	7	2	0.40	0.11
Sham operation								
19.5	6	232	2530	1120	170	72	0.43	0.12
19.5	6	282	2040	1300	318	110	0.26	0.12

obtained for labeled phosphatide content of plasma and tissues of normal dogs are stated in Table 120. Six hours after injection of labeled phosphate, the values for phosphatide ^{32}P per gram tissue phosphatide were about 100 times greater in kidney than in plasma. If a transfer to plasma from kidney and small intestine occurs, it must be, in contra-

TABLE 120

Recovery of Intraperitoneally Injected ³²P as Phosphatide ³²P in Plasma and Tissues of Normal Dogs[97]

Dog weight, kg.	Time after ³²P injection, hr.	Organ weight, g.			Phosphatide content at end of experiment, mg./100 g. tissue					Recovery of phosphatide ³²P as per cent of injected ³²P per g. phosphatide				
		Liver	Kidney	Small intestine	Plasma	Liver	Kidney	Small intestine	Muscle	Plasma	Liver	Kidney	Small intestine	Muscle
17.1	6	326	44	216	264	2730	2260	1350	850	0.13	0.37	0.25	0.14	0.0084
9.6	6	308	41	226	462	3000	2340	1330	1080	0.15	0.32	0.19	0.15	0.0061
15.0	18	284	57	198	341	2280	2100	1410	1010	0.47	0.53	0.29	0.33	
10.5	18	213	37	278	345	3000	2160	1070	1220	0.55	0.52	0.40	0.40	
9.8	18	217	42	200	357	3120	2950	1040	1070	0.33	0.34	0.21	0.20	0.015
18.7	18	315	49	265	360	2780	2200	1160	1160	0.30	0.32	0.21	0.20	0.0092
9.5	36	292	52	276	219	2060	2100	1220		0.54	0.56	0.44	0.33	
10.2	36	246	50	266	272	2930	2620	1050	1280	0.42	0.41	0.29	0.38	0.012
7.0	98	228	27	201	271	2490	2430	1250	970	0.28	0.26	0.27	0.21	0.10
7.0	98	243	32	136	435	2580	2090	1350	1150	0.30	0.29	0.32	0.26	0.043
6.0	98	225	46	190	357	2740	2000	1180	1060	0.41	0.36	0.40	0.35	0.55

distinction to transfer to plasma from liver, a slow process; this result was arrived at also in experiments in which plasma containing labeled phosphatides was injected into the circulation (see page 304). This result strongly supports the conclusion that plasma phosphatides are derived mainly from liver. In the blood of the fasting mouse, as is to be expected, the phosphatide phosphorus specific activity values fall into a pattern of change which is similar to that observed in liver phosphatides.[54]

Q. STUDY OF LECITHINEMIA

About 2 hours after administration of a meal containing fat, the fat content and phosphatide content of the blood begins to rise. A maximum is reached after 4 hours.[98] The increase in lecithin content of the plasma could be due to lecithin synthesized in the intestinal mucosa and absorbed into the blood or to mobilization of phosphatides synthesized in the liver or other organs.

The following experiment[99] shows that at least a large part of the phosphatide excess found in lipemic blood is not due to phosphatide molecules taken up from the intestine. At the start of an experiment 150 mg. labeled phosphorus as sodium phosphate was administered to a fasting dog weighing 7 kg., and another 150 mg. was given after the lapse of 2 hours. Simultaneously with the second sodium phosphate dose, 50 g. olive oil was fed. Six hours after the start of the experiment a pronounced lipemia was found to have taken place, and a rise of the blood phosphatide phosphorus amounting to 2.5 milligrams per cent was observed in this lipemic state. Of this 2.5 mg. per 100 ml., however, only 0.048 mg. per 100 ml. was labeled phosphatide phosphorus. The rest was nonlabeled phosphatide phosphorus mobilized by the liver or by other organs.

When interpreting these figures, it must be considered that the intestinal tract of the fasting dog contained endogenous phosphorus which "diluted" the labeled phosphorus fed to the dog, and, furthermore, that an exchange of plasma phosphatides with organ phosphatides takes place which will result in the replacement of a part of the labeled blood phosphatides by unlabeled organ phosphatides. But it was found that in the course of 2 hours less than half the plasma phosphatides was replaced by organ phosphatides (Table 117 and Hevesy[2]); thus renewal of labeled blood phosphatides during the experiment cannot explain the large difference found between the increment of total blood phosphatides and the increment of labeled blood phosphatides, the ratio of which

[98] W. R. Bloor, *J. Biol. Chem.*, **23**, 317 (1915).
[99] G. Hevesy and E. Lundsgaard, *Nature*, **140**, 275 (1937).

was as high as 31. Nor can the effect of the "dilution" by intestinal phosphate of the labeled phosphate fed to the animal explain more than part of the above-mentioned difference.

In connection with the above consideration, it is of interest to consider some results obtained by Cavanagh and Raper.[100] They found that the phosphatides extracted from the livers of rats six hours after the feeding of deuterium-containing linseed oil contained four times as much deuterium (in atoms per cent) as the phosphatides of the plasma. After a lapse of ten hours almost equal figures were obtained (compare page 321).

R. ORIGIN OF PHOSPHATIDES AND OTHER PHOSPHORUS COMPOUNDS OF YOLK

Prior to the use of labeled phosphorus, the question of site of formation of yolk material was far from settled, as will be seen from the following passage from a review of egg formation of the domestic fowl published in 1938 in *Physiological Reviews* by Conrad and Scott.

"Little is known of the mechanism of formation of this yolk material. There are three general ways in which yolk might be formed. The materials of the yolk might be synthesized elsewhere, carried into the ovary by the blood and deposited in the yolk. They might be formed in the follicular epithelial cells and secreted in the yolk. Finally, the yolk material might be synthesized in the yolk from nutrients reaching it through the follicular epithelium from the blood. Perhaps all three mechanisms have some part in the yolk formation."

A hen laying daily incorporates about 1.5 g. of phosphatide in the yolk, which corresponds to about 10% of the yolk weight. This percentage is much more than the amount contained in the daily food of the hen. Furthermore, it was found by different investigators that the fact that a fowl was raised on diets containing phosphorus in inorganic form only did not unfavorably influence its egg-laying capacity. It is, therefore, wholly or mainly in the organs of the hen that the synthesis of the phosphatide molecules of the yolk takes place. For the purpose of securing information regarding the organ in which the yolk phosphatide is primary synthesized, experiments were carried out by Hevesy and Hahn[101] with ^{32}P as an indicator.

Labeled sodium phosphate was administered to laying hens, the eggs laid were collected, and the specific activities of the phosphatide phosphorus extracted from

[100] B. Cavanagh and H. S. Raper, *Biochem. J.*, **33**, 17 (1939).

[101] G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **14**, No. 2 (1938).

the yolks were determined. In other experiments, the hen was killed and the specific activities of the phosphatide phosphorus of the yolks, ovary, liver, intestinal mucosa, and plasma were compared. The figures in the table below show the results obtained for the specific activity of phosphatide phosphorus extracted from different organs of a hen 5 hours after administration of labeled sodium phosphate.[101]

Organ	Relative specific activity
Liver..............................	100
Plasma............................	79
Ovary.............................	7.2
Yolk..............................	9.2
Intestinal mucosa...................	18

Ovary phosphatides are only slightly active; plasma phosphatides show a marked activity; and liver phosphatides show the greatest activity. Thus, the gradient in flow of labeled phosphatides is directed from plasma to ovary. The explanation suggests itself that yolk phosphatides are supplied by plasma phosphatides and that the role of the ovary, in supplying egg phosphatides, is to remove phosphatides from plasma and to incorporate them into yolk. Nature endowed plasma of birds actively engaged in egg laying with a much higher phosphatide content (about 25 milligram per cent) than is found in plasma of other animals or in plasma of male birds and immature females, obviously in order to facilitate passage of the large amounts of phosphatides which the plasma of the laying bird has to carry into the ovary. The total phosphatide content of the plasma of the laying hen in question amounted to 15 mg. The hen, laying daily, incorporated about 50 mg. phosphatide phosphorus into the yolk within 24 hours (nearly four times the phosphatide content of the plasma). The phosphatide content of the plasma was thus almost wholly renewed in the course of the 5 hours of the experiment.

The figures given above suggest, furthermore, the probability that phosphatide molecules of yolk are mainly synthesized in liver and are passed on by plasma to their destination. The liver contained 34 mg. of phosphatide phosphorus, and, since in the course of 5 hours some 9 mg. of phosphatide phosphorus was carried into the ovary, about one-fourth the liver phosphatides must have been renewed within 5 hours to supply the phosphatides incorporated into the yolks, a figure compatible with the results obtained in the investigation of rate of renewal of liver phosphatides (see page 279). The fact that the yolks

show but a small activity is due to the dilution of the strongly active phosphatides incorporated during the last 5 hours by the large amounts of nonlabeled phosphatides already present in the yolk.

Within the yolk, no new formation of phosphatides (no formation of labeled phosphatides) takes place. This is shown by the fact that, if the labeled phosphate is administered after the egg has left the ovary, no active phosphatides are found in the yolk, as distinct from active inorganic phosphate which penetrates from the circulation into the egg during every phase of its formation.

The same fact is borne out by experiments *in vitro* in which eggs were placed for one day in a solution containing active phosphate. Of the activity found in the eggs, 99.4% was located in the shell, 0.4% in the white, and 0.2% in the yolk. The phosphatides extracted from the yolk were found to be inactive.

TABLE 121

Labeled Phosphorus Content of Eggs[101]

Time between administration of active P and egg laying	Per cent labeled P administered found in			
	Shell	Albumin	Total yolk	Yolk lecithin
5 hours	0.24	0.0015	0.0014	0.000
1.0 days	0.052	0.032	0.109	0.014
3.0 days	0.036	0.030	0.42	0.17
4.5 days	0.026	0.027	0.95	0.34
6.5 days	0.022	0.020	0.85	0.35

The egg enters the oviduct about 15 minutes after ovulation, passes through the funnel in 18 minutes, spends 3 hours in traversing the magnum or albumin-secreting portion of the oviduct, 1 hour in the isthmus, and the remainder of the period (usually 20 – 24 hours) in the uterus. Thus, the egg spends about one day outside the ovary before being laid. When labeled phosphate was administered to a hen 5 hours before laying, the ovum was certainly no longer in the ovary. This egg (see Table 121) did not contain active phosphatides, as was to be expected. Active non-phosphatide (mainly inorganic) phosphorus was found in the yolk, however, and the phosphorus of the white and the shell also showed very high activity.

Shell deposition begins practically as soon as the egg reaches the uterus and presumably continues until oviposition. During the time

the egg is in the uterus, approximately 5 g. of calcium carbonate, containing a small amount of phosphate (3–4 mg. phosphorus), is deposited in the shell membranes as the egg shell. This phosphate is secreted from a plasma containing highly active phosphate shortly after the administration of ^{32}P, and the shell phosphate secreted shortly after administration of labeled phosphate is bound to be highly active. In the course of the next few days the activity of the plasma phosphate decreases and the shell of the eggs laid after a day or more is found to be less and less active, as seen in Table 121.

Lorenz, Perlman, and Chaikoff[102] showed that the amount of ^{32}P, deposited in phosphatides and other compounds in the yolk could be accounted for by an integral function of the two variables, yolk growth rate and ^{32}P availability, during the corresponding period of new formation. These experiments also showed a marked dissimilarity in the deposition of phosphorus in shell and in albumin. Those shells that were being actively formed at the time of injection showed a high ^{32}P content (up to 0.24% of the amount injected), whereas eggs that entered the uterus several hours later contained much smaller amounts of ^{32}P in their shells. Albumin protein is secreted at the time the egg is in the magnum and its deposition is completed about twenty-two hours

TABLE 122

Per Cent [32]P Administered as Phosphate Found in Phosphatides of Laying and Nonlaying Birds[103]

Organ	Laying bird		Nonlaying bird	
	6 hr.	12 hr.	6 hr.	12 hr.
Entire bird..................	3.62	4.55	3.25	4.57
Gastrointestinal tract..........	10	10	23	15
Muscle + bone + blood........	32	36	27	35
Ovary + oviduct + yolks	11[a]	20	0.4	0.2
Liver.......................	44	29	47	44

[a] 10% in yolk, and only 1% in ovary and oviduct.

before oviposition. Eggs laid during the 24- to 30-hour interval entered the magnum at a time when plasma radiophosphate was at its maximum.

[102] F. W. Lorenz, I. Perlman, and I. L. Chaikoff, *Am. J. Physiol.*, **138**, 318 (1942–1943).

[103] C. Entenman, S. Ruben, I. Perlman, F. W. Lorenz, and I. L. Chaikoff, *J. Biol. Chem.*, **124**, 795 (1938).

The ^{32}P content in the albumin of these eggs did not exceed 0.05%, whereas eggs laid between 45 and 75 hours contained 2–4 times this amount. The delayed deposition of ^{32}P in the albumin (see also Hevesy and Hahn[101]) suggests that a synthetic process precedes the deposition of phosphorus-containing compounds. Egg albumin is known to contain slight amounts of phosphorus and it is not unlikely that the delay is due to the incorporation of phosphorus into this or other proteins before their deposition in the albumin.

Chaikoff and associates[103] determined what percentage of ^{32}P administered was present as phosphatide phosphorus in the laying and the nonlaying bird. The results are given in Table 122, which shows that nearly one-half the amount of active phosphatides of the bird is located in the liver, although the phosphatide content of the liver may be estimated to represent but 5% of that of the bird.

S. EFFECT OF DIETHYLSTILBESTROL ON TURNOVER OF PHOSPHATIDES

Flock and Bollman[104] investigated phosphatide turnover following administration of diethylstilbestrol to cocks. When radioactive sodium phosphate was given intraperitoneally to cocks, labeled phosphatides appeared in the plasma in 2 hours and increased at an uniform rate for 12 hours. Six hours after the administration of ^{32}P the specific activity of the phosphatide of plasma was similar in cocks which received diethylstilbestrol and in untreated birds. The concentration of phosphatides was, however, greater in plasma of treated than in plasma of untreated birds, and the total ^{32}P content was correspondingly greater.

Calculations based on the ^{32}P content of liver phosphatides and the amount and ^{32}P content of phosphatides of plasma indicate that 1.51 mg. of phosphatide phosphorus entered each 100 ml. of plasma every hour in the untreated birds. Similar calculations showed an average of 5.0 mg. entering each hour in birds which had received diethylstilbestrol.

Calculations based on the disappearance of radioactive phosphatides from plasma of normal birds after intravenous injection of radioactive phosphatides into plasma indicate that 1.47 mg. of phosphatide phosphorus left each 100 ml. of plasma every hour. An average of 2.76 mg. left the plasma each hour in birds which had received diethylstilbestrol. Administration of diethylstilbestrol to birds appears to increase the rate of formation of phosphatides and also the rate at which they leave

[104] E. V. Flock and J. L. Bollman, *J. Biol. Chem.*, **156**, 151 (1944).

the plasma. The rate of phosphatide formation in the livers of cocks has been shown to be increased by the administration of stilbestrol by Taurog et al.[105] They compared the formation of labeled phosphatides in surviving liver slices of cocks treated with 10–15 mg. diethylstilbestrol with phosphatide formation in controls, and observed a significant increase in formation in the stilbestrol-treated birds.

T. TURNOVER OF VITELLIN

In an investigation carried out by Chargaff,[106] the ^{32}P content of the phosphorus of "free" lecithin and cephalin, of the "combined" phosphatides accompanying the vitellin fraction, and of the vitellin fraction itself were investigated. While about 50% of the phosphatides present in yolk, the "free" phosphatides, can be extracted with ether, the remainder, the "combined" phosphatides, are present as a constituent of the lipide–protein complex, lipovitellin, contained in hen egg yolk.

Phosphorus compounds isolated from yolks of eggs laid in the course of 8 days following intramuscular injection of radioactive sodium phosphate were examined individually. Each of the hens received two intramuscular injections. The second injections were given 18 hours after the first. The specific activities of the phosphorus extracted from the fractions, and hence the rate of formation of "free" lecithin and cephalin and of the "combined" phosphatides accompanying the vitellin fraction, were found to be equal. Vitellin phosphorus, however, exhibited a considerably higher specific activity in the first 5 to 6 days of the experiment than the phosphatide phosphorus. The maximum specific activity was obtained for all fractions after a lapse of 6 days (see Fig. 62).

The higher activity of vitellin can be explained by assuming that phosphatides were formed at a slower rate than vitellin, and, correspondingly, that vitellin was formed in the first part of the experiment with the participation of more highly active phosphorus than were the phosphatides. If this explanation is correct, we should expect the vitellin phosphorus in the later phases of the experiment to be less active than the phosphatide phosphorus. This is actually found to be the case: after the lapse of 8 days the vitellin phosphorus was found to be somewhat less active than the phosphatide phosphorus.

[105] A. Taurog, F. W. Lorenz, C. Entenman, and I. L. Chaikoff, *Endocrinology*, **35**, 483 (1944).

[106] E. Chargaff, *J. Biol. Chem.*, **142**, 505 (1942).

An additional reason for the higher activity of vitellin phosphorus in the first phase of the experiment may be sought in a greater dilution of the active phosphatides by the nonactive (old) phosphatides present in the organism. Substantial amounts of phosphatides are present in
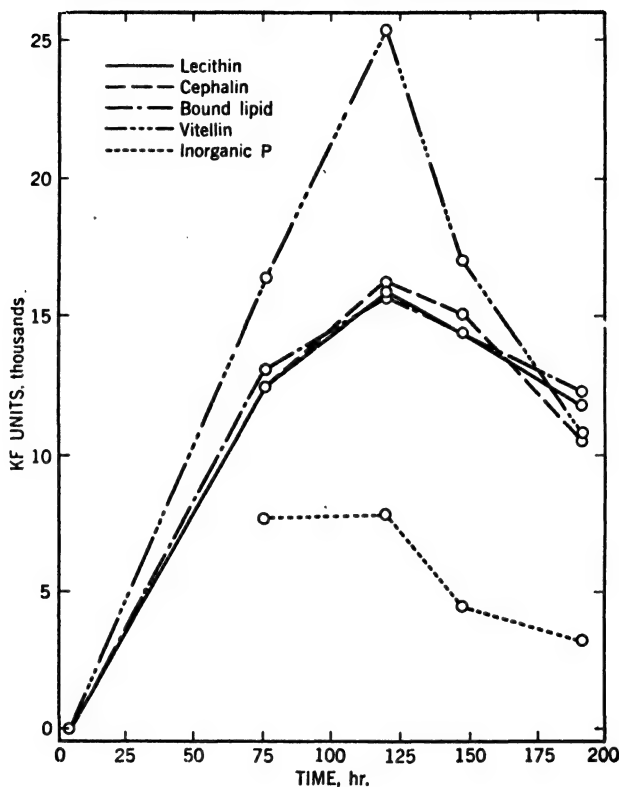
Fig. 62. Concentration of radioactive phosphorus in yolk fractions.[106]

the organism, and part of these interchange with the plasma phosphatides. The latter will consequently be responsible for diluting the active phosphatides. On the other hand, vitellin is present only in small amounts in the hen, being readily detectable only in the blood of laying hens.[107]

The discovery of the presence of the hydroxyamino acid serine in brain phosphatides led Chargaff to suggest that a metabolic link existed between phosphoproteins and phosphatides, the mechanism possibly

[107] R. R. Roepke and L. D. Bushnell, *J. Immunol.*, **30**, 109 (1936).

proceeding in the following steps: phosphorylation of the protein at the serine hydroxyl group, esterification of the phosphoprotein with diglycerides, decarboxylation of the latter to the corresponding ethanolamine-containing cephalins, etc. The above result, according to which the vitellin fraction contained more labeled phosphorus than the phosphatide fraction, can be interpreted as favoring this hypothesis.

U. ORIGIN OF PHOSPHATIDES IN CHICK EMBRYO

Only about two-thirds of the phosphatides in the egg are hydrolyzed during its incubation. Considering the large store of phosphatides in yolk (even shortly before the egg is hatched), we should expect the embryo to avail itself of this store when it needs phosphatides to build up its nervous system and other organs containing these substances. This point may be checked by introducing a small amount of labeled sodium phosphate, dissolved in 0.1 ml. of physiological sodium chloride solution, into the white of the egg before incubation, and determining whether, and to what extent, the phosphatides of the yolk and the embryo become labeled.[108] If none of the phosphatides are labeled,

TABLE 123

Relative Specific Activity of Phosphatides Extracted from Embryo
and Residual Yolk[108]

Time of incubation, days	Phosphatides extracted	Specific activity
6	Yolk	0.032
	Embryo	100
11	Yolk	0.10
	Embryo	100
18	Yolk	0.92
	Embryo	100

we may conclude that the phosphatide molecules in the embryo are not newly synthesized from inorganic phosphate present there. If, on the other hand, the yolk phosphatide remains unlabeled while that of the embryo becomes radioactive, we may conclude that the phosphatide molecules in the embryo have not come from the yolk but have been built up in the embryo with the participation of labeled inorganic phosphorus. As seen in Table 123, phosphatides extracted from the

[108] G. Hevesy, H. Levi, and O. Rebbe, *Biochem. J.*, **32**, 2147 (1938).

embryo invariably showed a high specific activity, while those from the yolk were barely active. The slight activity of yolk phosphatide, which increases with age of the embryo, is possibly due to an influx from the embryo into the yolk of a small amount of labeled phosphatides or of the enzymes responsible for resynthesis of phosphatides. The behavior of yolk phosphatides during incubation also illustrates the point discussed above, namely, that labeled phosphatides found in the yolk must have been deposited as such and that phosphatides once incorporated into the yolk cannot become labeled.

The specific activities of inorganic phosphorus, hexose monophosphate phosphorus, creatine phosphorus, phosphatide phosphorus, and residual phosphorus extracted from the embryo had the same value, showing that the inorganic phosphorus atom reaching the embryo has the same chance of entering a phosphatide or other molecule.

V. ORIGIN OF MILK PHOSPHATIDES

The origin of phosphatides of goat milk[109,110] was investigated along similar lines to studies of the origin of yolk phosphatides. Phosphatides extracted from milk a few hours after subcutaneous injection of labeled phosphate were found to be much more active than those present in the plasma. Thus, phosphatide molecules of the milk cannot have originated (compare Aylward[111]) in the plasma, but must have been built up mainly in the mammary gland. The specific activity of the phosphatide phosphorus extracted from the gland was, in fact, found to be higher than that of the corresponding products from the plasma and the milk, as is seen in Table 124.

Phosphatides are renewed in the milk gland at a still higher rate than in the liver. Investigation of the activity of different milk fractions by Aten[109] proved that no mixing occurred in the milk while stored in the udder. Moreover, it was found that, a few hours after the start of the experiment, the specific activity of the phosphorus of casein and of acid-soluble organic phosphorus compounds was but slightly lower than that of inorganic phosphate of the milk. This fact makes it seem very probable that these substances are formed in the milk gland from

[109] A. H. W. Aten, Jr., "Isotopes and Formation of Milk and Egg," *Dissertation*, Utrecht, 1939.

[110] A. H. W. Aten, Jr., and G. Hevesy, *Nature*, **142**, 111 (1938).

[111] F. X. Aylward, J. H. Blackwood, and J. A. B. Smith, *Biochem. J.*, **31**, 130 (1937).

inorganic phosphate. The phosphorus atom is found to require 0.5 to 2.5 hours more to enter milk casein than to enter milk phosphate. The corresponding time for its entry into milk phosphatides is over 2 days.

TABLE 124

Activity of Phosphatide Phosphorus of Milk and Organs of a Goat[109]

Fraction	Relative specific activity[a] per mg. phosphatide P
Milk....................................	1
Plasma..................................	0.02
Milk gland..............................	1.4
Liver...................................	1
Kidneys.................................	1.2

[a] 4.5 hr. after administration of labeled sodium phosphate.

Kay and colleagues[112] have taken blood samples at different times after milking from the internal iliac artery and from the abdominal subcutaneous (mammary) vein of the cow and have determined phosphatide phosphorus, ester phosphorus, inorganic phosphorus, fatty acid, and other contents of these samples and of the corresponding plasma samples. No significant difference was found in content of phosphatide phosphorus and ester phosphorus, while inorganic phosphorus content of the plasma of the mammary vein was found to average 7%, and fatty acid content 2%, lower than the corresponding values for the iliac artery. From these findings it was concluded that the fat of cows' milk was derived in the main from nonphosphatide fatty acids of blood, *i.e.*, probably from the fatty acids of neutral fat. Phosphatides are thus not the blood precursors of milk fat. Furthermore, it was concluded that phosphorus compounds of milk are mainly derived from inorganic phosphate of the blood plasma. These conclusions are in good accord with results arrived at by application of labeled phosphate to the study of precursors of phosphorus compounds in milk.

W.   TURNOVER OF PHOSPHATIDES IN CELL NUCLEI
EFFECT OF RÖNTGEN RAYS ON PHOSPHATIDE TURNOVER

Cell nuclei contain appreciable amounts of phosphatides. About 12% of the dry nuclei of rat liver is composed of phosphatides; the

[112] W. R. Graham, Jr., T. S. G. Jones, and H. D. Kay, *Proc. Roy. Soc. London,* **B120,** 330 (1936).

corresponding value for dry liver tissue is 8. In hepatoma and adeno-
carcinoma of the rat, 8% and 7% are given for the phosphatide content
of dry nuclei and dry tissue, respectively.[113] Other authors state that
phosphatide content of rat liver nuclei varies between 7.5% and
10.8%.[114] Since nuclei make up but a minor part of wet tissue, the
greater part of the phosphatides is located in cytoplasm.

TABLE 125

Effect of Röntgen Rays on Turnover of Phosphatides of Cell Nuclei
and Total Tissue in Jensen Sarcoma and Rat Liver[115]

| Groups of 12 rats of 150-g. av. wt. | Activity of 1 mg. phosphatide P as per cent of activity of 1 mg. inorganic tissue P | | | |
| | Sarcoma | | Liver | |
	Nuclei	Tissue	Nuclei	Tissue
Controls	2.86	3.75	10.34	20.44
Controls	2.38	2.37	8.15	11.25
Controls	2.75	2.67	11.33	14.25
Irradiated	2.56	2.32	3.29	12.30
Irradiated	1.02	1.52	4.08	8.91
Irradiated	1.44	1.41	4.83	8.20
Per cent decrease due to irradiation	37	38	59	36

TABLE 126

Effect of Röntgen Rays on Activity of Phosphatide Phosphorus of Nuclei
and Tissue of Rat Liver and Sarcoma[115]

| Animals | Activity of 1 mg. phosphatide P of rat sarcoma and liver as per cent of activity of 1 mg. inorganic P of blood plasma | | | |
| | Sarcoma | | Liver | |
	Nuclei	Tissue	Nuclei	Tissue
Controls	3.14	4.07	10.34	20.44
Controls	2.73	2.71	8.15	11.25
Controls	3.30	3.20	11.33	14.25
Irradiated	2.23	2.04	3.29	12.30
Irradiated	0.99	1.48	4.08	8.91
Irradiated	1.28	1.25	4.83	8.20
Per cent decrease due to irradiation	50	52	60	36

[113] H. H. Williams, M. Kaucher, A. J. Richards, E. Z. Moyer, and G. R. Sharp-
less, *J. Biol. Chem.*, **160**, 227 (1945).
[114] A. L. Dounce, *J. Biol. Chem.*, **151**, 221 (1943).
[115] G. Hevesy, *Nature*, **158**, 268 (1946).

Turnover rates of phosphatides in sarcoma[115] show no pronounced difference between rates of renewal of phosphatides in nuclei and in tissue, and correspondingly in the tissue composed mainly of cytoplasm. In nuclei of liver, however, rate of renewal of phosphatides clearly lags behind the rapid rate of turnover of these compounds in cytoplasm, as is seen in Table 125. To what extent failure to detect appreciable differences between turnover of phosphatides in nuclei and in tissue of sarcoma is due to a relatively rapid interchange between phosphatide molecules of cytoplasm and those of nuclei is not yet elucidated.

The effect of Röntgen rays on turnover rate of phosphatides present both in tissue and in nuclei was investigated as well. Two groups of twelve rats, after irradiation with 1000 r, are given labeled phosphate, while nonirradiated, control groups are treated in a similar way. After the lapse of 2 hours the animals are sacrificed and the sarcoma and livers are pooled separately. An aliquot is used in the determination of specific activities of inorganic and phosphatide phosphorus of the tissue, while from the bulk of the material cell nuclei are isolated by the method of Dounce.[114] The specific activities of the corresponding phosphorus fractions of the nuclei are also determined, and furthermore the activity of the inorganic phosphorus of the pooled blood plasma is measured. As seen in Tables 125 and 126, the rate of turnover of phosphatides in liver nuclei is markedly diminished by the action of Röntgen rays; that of the cytoplasm is also diminished though to a lesser extent.

X. APPLICATION OF INDICATORS OTHER THAN RADIOPHOSPHORUS IN STUDY OF PHOSPHATIDE METABOLISM

1. Incorporation of Deuterium-Containing Fatty Acids

Cavanagh and Raper[116] fed rats labeled fat which had been prepared by the partial saturation of unboiled linseed oils with deuterium. Deuterium comprised $4-5\%$ of the hydrogen atoms of the fat. The deuterium contents of phosphatides extracted from various tissues of the rats were then determined at various times. The presence of deuterium-containing phosphatides in liver and other organs a few hours after feeding the linseed oil is shown in Table 127.

At all intervals the highest value for deuterium content were observed in liver phosphatides. The smallest incorporation of deuterium was

[116] B. Cavanagh and H. S. Raper, *Biochem. J.*, **33**, 17 (1939).

observed in brain phosphatides, and thus the result obtained was similar to results of the incorporation of ^{32}P into phosphatides of different organs. The observation that plasma phosphatides have lower deuterium values than liver at the end of six hours was interpreted as suggesting incorporation of deuterated fatty acids into liver phosphatide. A similar conclusion was drawn concerning incorporation of phosphate into the phosphatide molecule (see page 309).

TABLE 127

Deuterium Content of Phosphatide Fractions after Feeding Fat
Containing 4–5 Atom Per Cent Deuterium[100]

	Atom per cent D after		
Organ	6 hours	10 hours	24 hours
Plasma.........	0.12	0.47	——
Liver..........	0.47	0.52	0.42
Kidney........	0.16	0.14	0.17
Brain	0.004	0.08	0.03

After keeping mice on a stock diet containing 20% deuterated linseed oil for three days, the liver phosphatide fatty acids were found, by Barrett and associates,[117] to contain nearly three times as much deuterium as body phosphatides.

2. Change in Degree of Unsaturation of Fatty Acids

Since phosphatides contain both saturated and unsaturated fatty acids, change in composition of the fatty acids of the organ phosphatides after ingestion of cod liver oil, for example, can be utilized in securing information regarding rate of phosphatide renewal in the organ in question. A change in iodine number of phosphatides extracted from the livers of dogs[118] and cats[119] after ingestion of cod liver oil, and disappearance of the changes within 24 hours and 2–3 days, respectively, have been observed in early experiments.

[117] H. M. Barrett, C. H. Best, and J. H. Ridout, *J. Physiol.*, **93**, 367 (1938).

[118] G. Ivánowics and E. P. Pick, *Wien. Klin. Wochschr.*, **23**, 573 (1910).

[119] R. G. Sinclair, *J. Biol. Chem.*, **82**, 117 (1929). See also R. G. Sinclair, *Physiol. Revs.*, **14**, 351 (1934).

3. Incorporation of Iodinated Fatty Acids

Iodinated fatty acids, whether injected intravenously or given by mouth, enter the phosphatides of liver, blood,[120] and milk,[121] for example.

4. Incorporation of Elaidic Acid

This method has been used repeatedly by Sinclair and others in the investigation of rate of renewal of phosphatides.[121a,122] Rate of entry of elaidic acid into and disappearance from phosphatides was found to be rapid in liver and intestinal mucosa and comparatively slow in muscle. The process was found to be essentially complete in liver within a day, but in muscle only after the period of many days.[80,122a]

Administered elaidic acid was found by Sinclair et al.[122] to appear early in plasma phosphatides of the cat. Rapid replacement of phosphatide fatty acids of plasma is indicated by the observation that, 8 hours after the feeding of elaidic acid, about 20% of the plasma phosphatide fatty acids consisted of this acid. Maximum incorporation after feeding occurs in less than 1 day in small intestine and in slightly more than 2 days in liver. In kidney and muscles, one-half of the maximum incorporation occurs in about 3 days. The rate of entry of elaidic acid into phosphatides of carcinosarcoma 256 is slower than into liver phosphatides but faster than entry into muscle phosphatide,[123] a behavior also shown by labeled phosphate.

The fact that phosphatide fatty acids of different tissues can be replaced only partly by elaidic acid alone restricts the applicability of elaidic acid as indicator. Not more than 30% of phosphatide fatty acids of liver and of small intestine [123a] can be replaced by elaidic acid,[123b] and not more than 7% of those of brain and still less in testes can thus be replaced. Furthermore, in order to obtain quantitative

[120] C. Artom, *Arch. intern. physiol.*, **36**, 101 (1933). C. Artom and G. Peretti, *ibid.*, **36**, 351 (1933).

[121] F. X. Aylward, J. H. Blackwood, and J. A. B. Smith, *Biochem. J.*, **31**, 130 (1937).

[121a] R. G. Sinclair, *Biol. Symposia*, **5**, 82 (1941).

[122] R. G. Sinclair, *J. Biol. Chem.*, **111**, 261 (1935); **115**, 211 (1936); **118**, 131 (1937).

[122a] M. F. Kohl, *J. Biol. Chem.*, **126**, 709 (1938).

[123] F. L. Haven, *J. Biol. Chem.*, **118**, 111 (1937).

[123a] R. G. Sinclair and C. J. Smith, *J. Biol. Chem.*, **121**, 361 (1937).

[123b] R. G. Sinclair, *J. Biol. Chem.*, **134**, 71, 83, 89 (1940).

results from experiments carried out with elaidic acid as indicator, we should know, besides the elaidic acid content of the phosphatides, the elaidic acid content of the fatty acid mixture available for phosphatide synthesis in cells of the organ. Lack of knowledge of the concentration of the tracer in the precursor of the compound whose rate of formation we wish to determine is the greatest obstacle in the application of isotopic and nonisotopic indicators to determination of turnover rate.

5. Incorporation of Fatty Acids with a Characteristic Absorption Spectrum

Linoleic acid of corn is converted by prolonged saponification to a fatty acid containing conjugated double bonds with high spectral absorption. Such fatty acids can be identified by their characteristic absorption spectra.[124] Conjugated fatty acids, when administered by mouth, are incorporated in phosphatides of intestinal mucosa. About 6% of the phosphatide fatty acids are found to be replaced by labeled fatty acids after the lapse of 1 hour and a maximum of 15% after 8 hours.

Barnes and associates[124,124a] found that rates of entry of conjugated fatty acids into phosphatides of intestinal mucosa and liver were not impaired in the adrenalectomized rat. In fat-deficient rats, however, a decrease in incorporation of the labeled fatty acids took place. Introduction of the tagged fatty acids into phosphatides of intestinal mucosa can also be demonstrated in experiments with tissue slices.

6. Incorporation of Heavy Nitrogen (^{15}N) in Phosphatides

Three days after choline, containing ^{15}N, had been administered, 21% or more of the choline present as phosphatide in the whole animal was found to be replaced by isotopic dietary choline. The corresponding replacement figure for labeled ethanolamine was 28% or more. The choline content of liver is renewed at the highest rate. Renewal of the choline of the gastrointestinal tract is slower than that in liver, slowest replacement takes place in the brain.[125]

[124] E. S. Miller and G. O. Burr, *Proc. Soc. Exptl. Biol. Med.*, **36**, 726 (1937). E. S. Miller, R. H. Barnes, J. P. Kass, and G. O. Burr, *ibid.*, **41**, 485 (1939).

[124a] R. H. Barnes, E. S. Miller, and G. O. Burr, *Am. J. Physiol.*, **126**, 427 (1939); *J. Biol. Chem.*, **140**, 233, 773 (1941); and *Proc. Soc. Exptl. Biol. Med.*, **42**, 45 (1939).

[125] D. Stetten, Jr., *J. Biol. Chem.*, **142**, 629 (1942).

7. Incorporation into Phosphatides of Analogs of Choline in Which Arsenic Replaces Nitrogen

Arsenic can be detected in the lecithin fraction isolated from liver and brain of rats kept for 21 days on a diet containing arsenocholine chloride.[126] (Compare Channon et al.[126a])

We thus complete the review of different methods in which isotopic and nonisotopic indicators have so far been applied in the investigation of the rate of renewal of the phosphatide molecules. The application of these methods demonstrates that renewals of the phosphate group, the fatty acid, and the nitrogen base group of phosphatide molecules take place. Replacement in liver is most pronounced; that in brain most sluggish. We cannot, however, yet state whether the rates of renewal of these three components differ and, if so, to what extent. Nor is it possible yet to determine to what degree phosphorylation processes are involved in fat absorption.

IV. Turnover of Nucleic Acids

A. TURNOVER OF DESOXYRIBONUCLEIC ACID

Desoxyribonucleic acid is wholly or mainly confined to cell nuclei. Since the desoxyribonucleic acid content of cell nuclei increases and decreases in the various stages of mitosis, we can expect an appreciable turnover of nucleic acid to take place in growing tissue, and in such organs as well which secrete products containing desoxyribonucleic acid. To the latter belong, among others, thymus, spleen, and bone marrow.

Liver and kidney of mature animals show, as is to be expected, a very low rate of renewal of their desoxyribonucleic acid content. The percentage ratio of activity of 1 mg. desoxyribonucleic acid phosphorus and of 1 mg. inorganic phosphorus in experiments taking 2 hours is found to be about 0.1% both in liver[127-129] and in kidneys,[127,129] though

126 H. Waelsch, W. M. Sperry, and V. A. Stoyanoff, J. Biol. Chem., 135, 291 (1940); 140, 885 (1941).

126a H. J. Channon, A. P. Platt, J. V. Loach, and J. A. B. Smith, Biochem. J., 31, 2181 (1937).

127 L. Ahlström, H. v. Euler, and G. Hevesy, Arkiv Kemi Mineral. Geol., A19, No. 9 (1944).

128 E. Hammarsten and G. Hevesy, Acta Physiol. Scand., 11, 335 (1946).

129 G. Hevesy and J. Ottesen, Acta Physiol. Scand., 5, 237 (1943). See also L. Hahn and G. Hevesy, Nature, 145, 549 (1940).

in the liver in some cases appreciably higher values are obtained. The corresponding ratio is very much larger in the case of spleen and intestinal mucosa, as seen in Table 128 and Figure 63.

The highest rate of renewal is found, as shown by Andreasen and Ottesen,[130] in bone marrow and thymus gland of the rat.
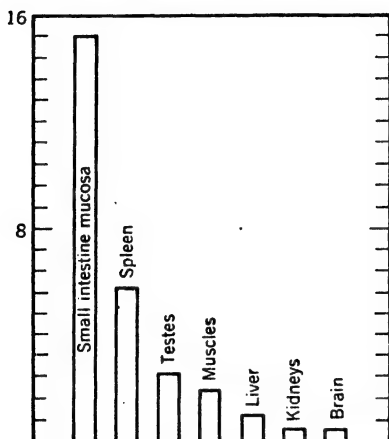
Fig. 63. Daily percentage renewal of desoxyribonucleic acid in organs of the full-grown rat.[129]

The data in Figure 63 are obtained by comparing the specific activity of desoxyribonucleic acid phosphorus at the end of the experiment with the average value prevailing throughout the experiment of the specific activity of inorganic phosphorus of the organ. It is thus assumed that the inorganic phosphate, or a phosphorus-containing precursor which attains relatively rapid equilibrium with inorganic phosphate, is incorporated in the newly formed desoxyribonucleic acid molecules.

Calculation of the above figures was made without regard to repeated renewal of some of the molecules during the experiment. The values stated for daily percentage renewal in intestinal mucosa and, to a still more restricted extent, in some other organs, may be correspondingly somewhat low.

In Table 128 the percentage ratios of specific activities of desoxyribonucleic acid phosphorus and of inorganic phosphate, both at the end of a 2-hour experiment, are recorded. The figures represent the average of numerous values obtained. The end value of specific activity of liver inorganic phosphate hardly differs from the mean value in experiments taking two hours. For spleen the end value is about 25% higher than the mean value.[38]

It can be assumed that much of the turnover of desoxyribonucleic acid takes place in dividing or secreting cells and it is quite possible that such cells are more permeable to phosphate than the average cell of the tissue in question. Such an enhanced permeability would not much influence the renewal figures obtained for liver desoxyribonucleic acid, as within a short time the specific activity of inorganic phosphorus of

[130] E. Andreasen and J. Ottesen, *Acta Path. Microbiol. Scand.*, **1944**, Suppl. LIV.

liver reaches the value in plasma. In the case of organs which show a restricted cell permeability, as for example in brain, testes, or muscle, in experiments taking 2 hours tissue inorganic phosphorus has, however, an appreciably lower specific activity than plasma inorganic phosphorus.

TABLE 128

Specific Activities of Desoxyribonucleic Acid Phosphorus and Inorganic Phosphorus Two Hours after Labeled Phosphate Administration to Mature Rat[38]

Organ	Percentage ratio of specific activities of desoxyribonucleic acid P and inorganic P
Liver	0.14
Spleen	2.50
Kidneys	0.16
Intestinal mucosa	4.80
Jensen sarcoma	2.11

In the case of these organs it is possible that desoxyribonucleic acid is synthesized from inorganic phosphorus having a higher specific activity than the inorganic phosphorus extracted from tissue; thus the renewal values arrived at are too high. The correct renewal figures, however, cannot be lower than the figures obtained by comparing the specific activity of desoxyribonucleic acid phosphorus of the organ with that of the inorganic phosphorus of plasma. The latter figures are given in Table 129. It is advisable in these, and also in other turnover experiments,[3b] to determine both the ratio of the specific activity of the phosphorus of the organic compound in question to the specific activity of tissue inorganic phosphorus and the ratio of the former to the specific

TABLE 129

Specific Activities of Desoxyribonucleic Acid P of Some Organs and That of Inorganic Phosphorus Plasma[38]

Organ	Percentage ratio of specific activities of 1 mg. desoxyribonucleic acid P and of 1 mg. plasma inorganic P
Kidney	0.15
Liver	0.20
Spleen	1.79
Intestinal mucosa	2.1
Jensen sarcoma	1.8

activity of plasma inorganic phosphorus. If the formation of the organic compound is preceded by an organic precursor, the ratio of the specific activities of the phosphorus of the final product and the precursor should be determined as well.

In the spontaneously retrograde Jensen sarcoma the percentage new formation is not found to be significantly lower than in the growing tumor. This finding indicates that the enzyme mechanism responsible for the incorporation of phosphate into the desoxyribonucleic acid molecule is not disturbed in the spontaneously regressive sarcoma. Treatment of rats with colchicine previous to the administration of labeled phosphate influences the extent of percentage renewal of desoxyribonucleic acid to a minor extent.[131]

Experiments in which the specific activity of desoxyribonucleic acid of liver was investigated several days after administration of labeled phosphate[132] are discussed on page 334. In surviving Jensen sarcoma tissue slices shaken with a Ringer solution containing labeled phosphate at 37°C., the formation of minute amounts of labeled desoxyribonucleic acid was found to take place.[133] Addition of cyanide, fluoride, or monoiodoacetate, but not of stilbestrol, caffeine, or colchicine, suppressed the formation of radioactive desoxyribonucleic acid.

Andreasen and Ottesen[133a] determined the percentage ratio of the injected amount of ^{32}P in 1 mg. desoxyribonucleic acid and 1 mg. inorganic plasma phosphate of lymphoid organs in experiments taking three hours in infant, young, and in old rats. Their results (average of 3 experiments) in Table 130 indicate a decrease with age of the animal in rate of formation of labeled desoxyribonucleic acid in thymus, lymph nodes, and spleen, the decrease being partly due to a decreasing rate of penetration of the radioactive tracer into the tissues. This rate of penetration for lymph nodes and spleen was found to be appreciably lower than for thymus.

In organs of growing rats, besides renewal of nucleic acid molecules, an appreciable additional formation of nucleic acid takes place, since the nucleic acid content of the organs increases with increasing weight.

[131] L. Ahlström, H. v. Euler, and G. Hevesy, *Arkiv Kemi Mineral. Geol.*, **A24**, No. 12 (1947).

[132] A. M. Brues, M. M. Tracy, and W. E. Cohn, *Science*, **95**, 558 (1942).

[133] L. Ahlström, H. v. Euler, and G. Hevesy, *Arkiv Kemi Mineral. Geol.*, **A21**, No. 6 (1945).

[133a] E. Andreasen and J. Ottesen, *Acta Physiol. Scand.*, **10**, 258 (1945).

That the percentage ratio of labeled, and thus newly formed, nucleic acid molecules is much larger in 3.5-day-old than in mature rats is seen by comparing the figures of Tables 128 and 131.[38]

TABLE 130

Desoxyribonucleic Acid Turnover in Lymphoid Organs of Rats of Different Age in Experiments Taking Three Hours[133a]

Organ	Percentage ratio of specific activity of nucleic acid P of organ and inorganic P of plasma		
	30-day old rats	110-day old rats	143-day old rats
Thymus....................	7.6	5.3	5.6
Lymph nodes of intestines	6.1	1.5	1.00
Lymph nodes of skin.........	2.9	1.4	1.1
Spleen.....................	2.4	1.6	0.8
Bone marrow...............	——	10	13

TABLE 131

Specific Activities of Nucleic Acid P and Inorganic P Two Hours after Administration of Labeled Phosphate to 3.5-Day-Old Rats[38]

Organ	Percentage ratio of specific activities of desoxyribonucleic acid P and inorganic P
Liver..	1.96
Spleen.......................................	9.76

The percentage increase in total desoxyribonucleic acid content of the liver of the 3.5-day-old rat amounts, in the course of 2 hours, to 0.9%. About half of the newly formed (labeled) desoxyribonucleic acid molecules present are due to increase in nucleic acid content of liver, while the other half are due to renewal of old molecules. A similar result was obtained in the investigation of formation of labeled nucleic acid in Jensen sarcoma. While in the course of 2 hours the increment in nucleic acid content of the sarcoma is found to average 1.5%, labeled nucleic acid molecules formed during this time amount to about twice this value.[134,135]

[134] H. v. Euler and G. Hevesy, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **17**, 8 (1942); *Arkiv Kemi Mineral. Geol.*, **A17**, No. 30 (1944).

[135] L. Ahlström, H. v. Euler, G. Hevesy, and K. Zerahn, *Arkiv Kemi Mineral. Geol.*, **A23**, No. 10 (1946).

It is of interest to compare rate of renewal of the phosphorus of desoxyribonucleic acid of liver with rate of renewal of liver proteins. Shemin and Rittenberg[136] found, by using ^{15}N as an indicator, that, in 7 days, half the liver protein was replaced by nitrogen from the diet and other proteins. Thus the rate of renewal of desoxyribonucleic acid of liver is about 20% of the renewal rate of liver proteins. Data on the renewal rate of purine nitrogen recently became available.[136a] In sarcoma slices incubated four hours at 37° C. in plasma containing labeled phosphate, about 0.2% of the desoxyribonucleic acid molecules are found to have been renewed.[133] In red corpuscles of the hen no renewal of appreciable amounts of desoxyribonucleic acid present takes place.[137]

In view of the low activity of desoxyribonucleic acid it is of great importance to purify very carefully the sample from other, more active phosphorus compounds present. Such a purification can be obtained by repeated solution in an alkaline medium and precipitation with a solution of hydrogen chloride in methanol.[138] By repeating these procedures 7 to 8 times, nucleic acid ^{32}P can be obtained with impurity of less than 1 part in 10,000.

In earlier investigations of turnover of nucleic acid, acid-soluble and phosphatide components of tissue were extracted with trichloroacetic acid and with ether – alcohol, and the activity of the residual part was determined. Such residues contain, beside desoxyribonucleic acid, ribonucleic acid, and also minor amounts of rapidly renewed phosphoproteins. Since the rate of renewal of ribonucleic acid is much larger than that of desoxyribonucleic acid, no conclusion about the value for desoxyribonucleic acid can be drawn from these experiments.

B. EFFECT OF RÖNTGEN RAYS ON TURNOVER OF DESOXYRIBONUCLEIC ACID

Röntgen radiation (100 r. or more) was found to decrease turnover rates of desoxyribonucleic acid, in both Jensen sarcoma and normal organs.[38,134,135,139] As seen in Table 132 the amount of newly formed desoxyribonucleic acid molecules, found to be 2 – 3% in the course of two hours in nonirradiated, growing sarcoma, is reduced by irradiation

[136] D. Shemin and D. Rittenberg, *J. Biol. Chem.*, **151**, 507 (1943).

[136a] G. B. Brown, P. M. Roll, A. A. Plentl, and L. F. Cavalieri, *J. Biol. Chem.*, **172**, 469 (1948).

[137] G. Hevesy and J. Ottesen, *Nature*, **156**, 534 (1945).

[138] H. B. Jones, *personal communication*.

[139] L. Ahlström, H. v. Euler, and G. Hevesy, *Arkiv Kemi Mineral. Geol.*, **A19**, No. 13 (1945).

to one-half to one-third of its normal value. These results are obtained in experiments in which the labeled phosphate is administered immediately after irradiation.

Röntgen radiation blocks the turnover of desoxyribonucleic acid molecules not only in growing tissue, but also in normal organs of adult animals,[38] as is seen in Table 132A.

TABLE 132

Effect of Irradiation of Jensen Rat Sarcoma on Formation of Desoxyribonucleic Acid[134]

Dosage, r.	Time between irradiation and injection	Time between injection and killing of rat, hr.	Ratio of newly formed nucleic acid in controls and in irradiated sarcoma
750–1500	Several minutes	0.5	3.2
335–1500	Several minutes	1	2.4
450–1500	Several minutes	2	2.2
1500	Several minutes	4–6	2.8
1230–1500	3 to 7 days	2	1.7

TABLE 132A

Ratio of Newly Formed Desoxyribonucleic Acid Molecules Present before and after Irradiation in the Organs of Adult Rats[38]

Organ	Ratio of newly formed nucleic acid in the organs of controls and of irradiated rats[a]
Liver	3.3
Spleen	2.4
Intestinal mucosa	2.3

[a] Dose applied 1480–3000 r. Rat irradiated *in toto.* [32]P injected after the irradiation. Rat killed two hours after administration of the [32]P.

The effect of irradiation on formation of nucleic acid molecules in organs of normal adult rats is thus similar in magnitude to the effect of Röntgen rays on the growing Jensen sarcoma (Table 132).

Röntgen rays were found to block formation of nucleic acid in organs of rapidly growing rats also. The percentage inhibition of the desoxyribonucleic acid turnover was found to be similar to that in normal organs of adult animals and in Jensen sarcoma. The ratio of newly formed desoxyribonucleic acid in organs of controls and in irradiated rats was found to be 2.3 in experiments taking two hours, in which the

3 to 4 day old rats were irradiated with 2000 – 2250 r. previous to administration of labeled phosphate.   The reaction of fundamental importance, the percentage formation of desoxyribonucleic acid, is thus influenced to a similar extent in all tissues investigated.   But the consequence of these disturbances by irradiation are very different in the growing than in the fully grown tissues.   Presumably this difference is at least to some extent due to the fact that, in the fully grown tissue, cells have plenty of time to recover before reaching the mitotic stage, while this is not the case in the rapidly growing tissues.[139a]

Irradiation throughout the experiment was found to reduce formation of labeled desoxyribonucleic acid to a still greater extent than that stated above.[140]   In these experiments, however, when the rats were fixed to a table and irradiated after injection of labeled phosphate, the low ^{32}P content of desoxyribonucleic acid was to a large extent due to disturbed circulation, which prevented the isotopic tracer from reaching the sarcoma cells.[141]

This disturbance is also demonstrated by the fact that the ratio of the specific activity of the inorganic phosphorus of the sarcoma and the plasma is much lower in these than in the usual experiments.   If the ratio between the specific activity of the inorganic phosphorus of the tissue and the plasma is low, the specific activity of the inorganic phosphorus of the tissue and of the tissue cells can differ appreciably, thus the ratio of the specific activity of nucleic acid phosphorus and tissue inorganic phosphorus no longer indicates the extent of new formation of nucleic acid.   The muscular strain exercised by the rat fixed to a table can furthermore diminish the rate of absorption of labeled phosphate injected with the result that the actual time of the experiment is shorter than the time between administration of the labeled phosphate and sacrificing the rat.[142]

In experiments with rats bearing two inoculated sarcomata, one sarcoma was irradiated while the other was effectively shielded.   A reduction in labeled nucleic acid formation took place in both, but the reduction in the shielded was smaller.[139]   An ionization chamber

[139a] G. Hevesy, *Rev. Modern Phys.*, **17**, 102 (1945).

[140] L. Ahlström, H. v. Euler, and G. Hevesy, *Arkiv Kemi Mineral. Geol.*, **B18**, No. 13 (1944).

[141] L. Ahlström, H. v. Euler, and G. Hevesy, *Arkiv Kemi Mineral. Geol.*, **A24**, No. 12 (1947).

[142] G. Hevesy, *Rev. Modern Phys.*, **17**, 102 (1945).

immersed in the shielded sarcoma indicated a dose of a few r. units only. This finding led to the following investigation.[135] Rabbits were irradiated with 10,000 r. and the irradiation was followed by blood transfusion from the irradiated to a nonirradiated sister rabbit. Blood from nonirradiated controls was also transfused to a sister rabbit. ^{32}P was then administered and the nucleic acid of the liver, kidneys, and intestinal mucosa was extracted. No difference was found between the rate of formation of labeled desoxyribonucleic acid of the rabbits which received irradiated and nonirradiated blood. No definite result was obtained in the investigation of liver desoxyribonucleic acid; while, in the case of kidneys, injection of irradiated blood diminished the rate of formation of desoxyribonucleic acid.

TABLE 133

Turnover of Desoxyribonucleic Acid in Two Sarcoma in the Same Rat, One Shielded, the Other Irradiated[139]

Organ	Activity of 1 mg. nucleic acid P, per cent of activity of 1 mg. inorganic P.^{a} from		
	Irradiated	Shielded	Nonirradiated controls
Sarcoma.............	0.85	1.00	1.89

^{a} Average figures from several experiments.

The possible existence[138] of an indirect effect of irradiation on the formation of desoxyribonucleic acid in the mammary carcinoma of the mouse was investigated by Jones. When radioactive chromic phosphate is injected into the mouse approximately 90% of the material is found[143] to be taken up by the liver, the bulk of the remaining fraction being found in the spleen and in the lungs. The accumulation of the active chromic phosphate brings about an intensity of β-radiation in the liver and spleen of the order of 100 times the concentration in other tissues.

Chromic phosphate suspension varying from 0.1 to 1.0 millicurie per mouse of radioactive phosphorus was injected intravenously. The animals were sacrificed when approximately 3400 r. had accumulated in the livers during a period of 4 to 48 hours. Two hours prior to the time of sacrifice, the animals were given a small amount of radioactive sodium phosphate (10 microcuries) intravenously. In all cases, independent of the time allowed for the accumulation of radiation, the results were the same, showing a depression of desoxyribonucleic acid synthesis in the tumor coincident with β-ray irradiation of the liver.[138]

[143] H. B. Jones, C. J. Wrobel, and W. R. Lyons, *J. Clin. Invest.*, **23**, 783 (1944).

C. RATE OF RENEWAL OF RIBONUCLEIC ACID

In contradistinction to desoxyribonucleic acid — the main constituent of cell nuclei — ribonucleic acid, which according to Brachet[144] and Caspersson[145,146] is generally found in cytoplasm, is renewed at a fairly rapid rate in liver and kidney. Hammarsten and Hevesy[147] found, for the percentage ratio of the specific activities of desoxyribonucleic acid phopshorus and inorganic phosphorus, and, for that of ribonucleic acid phosphorus and inorganic phosphorus, 2 hours after subcutaneous administration of labeled sodium phosphate, the values recorded in Table 134.

TABLE 134

Rate of Renewal of Ribonucleic and Desoxyribonucleic Acid in Organs of Rat Two Hours after Administration of Labeled Phosphate[147]

Organ	Nucleic acid	Percentage ratio of specific activity of nucleic acid P to that of inorganic P of organ	Ratio of specific activity of ribonucleic acid P to that of desoxyribonucleic acid P
Liver	Ribose	3.45	33
	Desoxyribose	0.105	
Spleen	Ribose	6.6	3
	Desoxyribose	2.2	
Intestine	Ribose	6.1	2
	Desoxyribose	2.8	

As Hammarsten finds, 70% of the nucleic acids of liver (a similar value of 75% was reported by Davidson and Waymouth[148]) and 44% of those of spleen are of the ribose type. In liver, in experiments taking 2 hours, 99% of the ^{32}P content of the nucleic acids turns out to be present in the ribose compound, the corresponding figure in the spleen being 70.

Brues and associates[149] determined the specific activity of both

[144] J. Brachet, *Compt. rend. soc. biol.*, **133**, 88 (1940).

[145] T. Caspersson and J. Schultz, *Nature*, **142**, 294 (1938); **143**, 602 (1939).

[146] T. Caspersson, *Skand. Arch. Physiol.*, Suppl. No. **8**, 73 (1936); *Chromosoma*, **1**, 147 (1940).

[147] E. Hammarsten and G. Hevesy, *Acta Physiol. Scand.*, **11**, 335 (1946).

[148] J. N. Davidson and C. Waymouth, *Biochem. J.*, **38**, 39 (1944).

[149] A. M. Brues, M. M. Tracy, and W. E. Cohn, *J. Biol. Chem.*, **155**, 619 (1944).

the ribonucleic acid and the desoxyribonucleic acid of resting and regenerating liver and also of hepatoma. In these experiments the labeled phosphate was administered several days before the rats were killed.

Both nucleoproteins were precipitated with calcium chloride and dexosyribonucleoprotein was separated by treatment with sodium chloride. Ribonucleic acid was purified by precipitation of the barium salt and subsequent precipitation of the free acid in g'acial acetic acid.

The results obtained, seen in Table 135, show that the rate of renewal of ribonucleic acid is higher than that of desoxyribonucleic acid and that turnover in regenerating liver and hepatoma is more rapid than in resting liver. That the ratio of specific activities of ribonucleic and desoxyribonucleic acid is found, in these experiments, to be appreciably smaller than in the experiments of Hammarsten and Hevesy, may be due, at least in part, to the much longer duration of the former experiments.

TABLE 135

Specific Activities of Nucleic Acid Phosphorus[149]

Organ	Time after injection, days	Nucleic acid, per cent of specific activity of inorganic P		Ratio
		Ribose	Desoxyribose	
Resting liver........	3	54.9	10.6	5.2
	8	123	20.8	5.9
Regenerating liver ..	3	230	180	1.3
	13	314	576	0.5
Hepatoma..........	3	171	64	2.7

Kohman and Rusch[150] administered labeled phosphate to rats and mice and determined the ^{32}P content of nucleoproteins (containing both desoxyribose and ribose compounds) of normal liver and of liver in which cancer was produced by feeding azo dyes. The tumorous liver, in which a rapid formation of new cells takes place, was found to have a 45% increase in uptake of ^{32}P, compared with the normal liver. ·

D. SPECIFIC ACTIVITY OF NUCLEIC ACID PHOSPHORUS
OF THE WHOLE RAT

Hammarsten and Hevesy[147] determined the specific activity of both total desoxyribonucleic acid phosphorus and total ribonucleic acid

[150] T. P. Kohman and H. P. Rusch, *Proc. Soc. Exptl. Biol. Med.*, **46**, 403 (1941).

phosphorus extracted from a rat weighing 194 g. The activity of labeled sodium phosphate administered amounted to 8.1 microcuries per 100 g. animal weight. The time of the experiment was 2 hours. The results of this experiment are shown in Table 136. As shown in the table, the

TABLE 136

Specific Activities of Nucleic Acid Phosphorus of Whole Rat, Liver, Spleen, and Intestinal Mucosa[147]

Sample	Specific activity (whole rat ribonucleic P = 100)		
	Ribonucleic	Desoxyribonucleic	Inorganic P
Total rat......	100	60	
Liver.........	164	4.4	5100
Spleen........	292	63	2850
Intestine......	112	63	2770

specific activity of the average nucleic acid phosphorus of the rat is almost identical with the value for ribo- and desoxyribonucleic acids, respectively, extracted from the intestine.

Interpretation of the significance of specific activity figures obtained for the whole rat poses some difficulties, since the specific activity of the inorganic phosphorus utilized in the formation of labeled nucleic acid molecules is unknown. If the specific activity of the inorganic phosphorus utilized in building up the average body nucleic acid were similar to the specific activity of inorganic liver phosphorus, the percentage rate of renewal of body ribo- and desoxyribonucleic acids would be 2.0 and 1.2, respectively. If the specific activity of inorganic phosphorus utilized in building up the average nucleic acid of the organism were similar to the specific activity of inorganic intestinal phosphorus, larger values, *i.e.*, 3.6 and 2.2, respectively, would be obtained.

It is improbable that inorganic phosphate of such great activity is utilized in synthesis of nucleic acid as is found in a 2-hour experiment in liver. Liver and kidneys have a privileged position in rate of uptake of phosphate. The amount of nucleic acid present in liver and kidneys makes up, furthermore, only a small percentage of total nucleic acid content of the organism. It is much more probable that inorganic phosphorus of similar specific activity as found in the intestine is used in building up the labeled nucleic acid molecules. In fact, the amount of nucleic acid present in the mucosa of the digestive tract makes up a large percentage of body nucleic acid. While body nucleic acid

contains slightly radioactive fractions, namely, those originating from liver, kidneys, and brain, and those fractions of restricted radioactivity originating from muscle, it contains also fractions of higher activity than found in the intestinal mucosa, namely, those originating from bone marrow and thymus. The lymphocytes secreted into the organism can also be expected to contain markedly active nucleic acid. This makes it understandable that the rate of renewal of average body nucleic acid corresponds to about the rate of renewal of intestinal nucleic acid and is thus considerable for both types of nucleic acid, in contradistinction to the rate of renewal found in liver, for example, which is very low for desoxyribonucleic acid and appreciably higher in the case of ribonucleic acid.

Andreasen and Ottesen[151] attempted to estimate lymphocyte production in different lymphoid organs from the rate of turnover of desoxyribonucleic acid in these organs. They concluded that the thymus must be the most important lymphocytopoietic organ except in old age.

The rate of formation of nucleic acid (especially of desoxyribonucleic acid in full-grown liver and some other organs) is slow, compared with the rate of formation of other phosphorus compounds. Correspondingly ^{32}P is lost by the nucleic acid fractions at a slower rate than from other compounds in the later phases of the experiment in which the gradient of the ^{32}P content of the inorganic phosphorus is directed from the tissues into the plasma. If the labeled phosphate is administered at the start of the experiment, which is the usual case, the specific activity in the later parts of protracted experiments of the inorganic phosphorus of the organs successively decreases and thus the renewal of organic phosphorus compounds occurs with the participation of less and less active inorganic phosphorus. In these phases of the experiment, the stream of ^{32}P will be directed from the organic compounds into the inorganic phosphate. Due to this fact, prolonging the time of the experiment over a certain time — over 2 days, for example, in the case of the Jensen sarcoma — will be disadvantageous for the yield of labeled desoxyribonucleic acid extracted from the organs. A loss of ^{32}P from an organic compound in an intact organ can only take place when the compound is not inert but is renewed. For example, the avian red corpuscles formed in the marrow of a labeled organism and released into the circulation, with increasing time, lose some of their ^{32}P content.

[151] E. Andreasen and J. Ottesen, *Acta Path. Microbiol. Scand.*, Supplement LIV (1944).

The desoxyribonucleic acid in the avian red corpuscle, not being renewed, retains its original ^{32}P content throughout its life cycle, in contrast to the other organic compounds of the corpuscle. Labeled yeast, when placed in an inactive nutritive solution and kept under completely anerobic conditions, under which no budding or increase in protein nitrogen is observed but where intense fermentation takes place, was found by Spiegelman and Kamen[152] to lose an appreciable part of its ^{32}P while no loss in the ^{32}P of the residual phosphorus was observed. In these experiments the ^{32}P of the residual phosphorus was presumably to a large extent present as ribonucleic acid phosphorus. When ammonia was added, budding occurred with a consequent turnover of residual phosphorus. This turnover, taking place in a medium of lower specific activity than shown by the nucleic acid phosphorus, led to loss of ^{32}P by the residual phosphorus fraction. These results indicate a very low turnover rate of ribonucleic acid in nondividing yeast. In resting yeast the turnover of ribosenucleic acid in the absence of nitrogen was found by Spiegelman and Kamen [152] to be quite low and much less than in other phosphate fractions. In the presence of nitrogen in sufficient amount (as ammonium sulfate) to induce assimilation but not growth, the turnover in nucleic acid was increased eightfold which was much more than the increase of almost any other fraction examined, with the possible exception of protein-bound metaphosphate.

V. Phosphorus Turnover in Cell Nuclei

Marshak[153,154] has isolated nuclei from liver and lymphoma tissue of rats weighing 150 g., and has shown that most ^{32}P taken up by the nuclei is present in the residue obtained after extraction with trichloroacetic acid and an ether–alcohol mixture. The phosphorus compound of the residue is presumably exclusively nucleoprotein. The distribution of ^{32}P in the nuclei is seen in Table 137.

Chemical analysis of the phosphorus of the liver nuclei shows that the ^{31}P content of the residual fraction makes up 81% of the total phosphorus content, the corresponding value for phosphatide, acid-soluble, and water-soluble fraction being 7, 6, and 7%, respectively. The residues of the tumor nuclei contain $90 - 95\%$ of the total nuclear activity at all times until the seventh day after injection of the labeled

[152] S. Spiegelman and M. D. Kamen, *Science*, **104**, 581 (1946).

[153] A. Marshak, *Science*, **92**, 460 (1940).

[154] A. Marshak, *J. Gen. Physiol.*, **25**, 275 (1941).

phosphate. Similar results were obtained in the investigation of ^{32}P uptake by nuclei of sarcoma 180.

TABLE 137

Distribution of ^{32}P in Cell Nuclei[154]

Organ	Time after injection	^{32}P distribution in nuclei, %				
		Water soluble	Acid soluble	Phosphatide	Residue	Total
Liver.........	1 hr.		6.0		67.2	94.6
	1 day	15.2	3.3	6.1	68.3	92.9
	3 days	26.1	0.8	4.7	66.0	97.6
	5 days	2.7	1.9	8.6	66.9	80.1
	7 days	12.6	7.7	7.7	71.6	99.6
Lymphoma ...	1 day	2.5	0.6	0.6	94.8	98.5
	2 days	1.5	0.7	2.3	95.4	99.9
	3 days	3.6	2.5	0.8	94.5	101.4
	5 days	2.1	3.4	0.3	88.1	93.9
	7 days	8.4	26.4	1.4	70.4	106.6

That ^{32}P accumulates in nuclei of rapidly growing tumor to a much greater extent than in fully grown liver is demonstrated by Figures 64
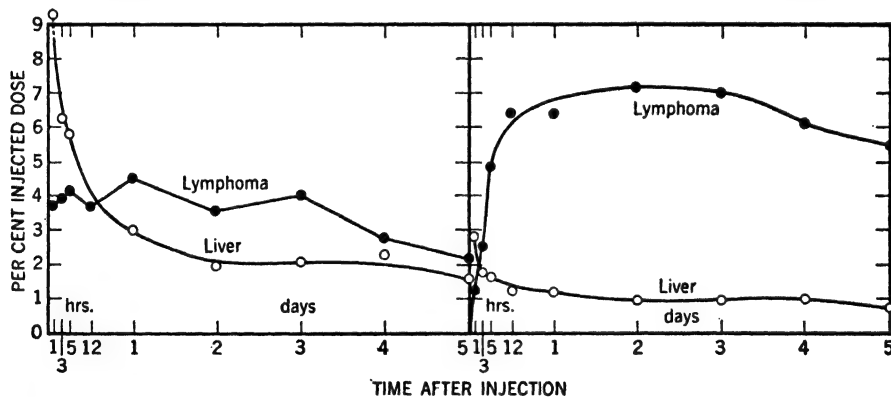
Fig. 64. ^{32}P uptake by tissues.[154]   Fig. 65. ^{32}P uptake by nuclei.[154]

to 66. This difference in accumulation of ^{32}P in nuclei of liver and of lymphoma is to be expected in view of the fact that most of the phosphorus of nuclei is present as desoxyribonucleic acid phosphorus, and that turnover of this compound is exceedingly low in nuclei of fully

grown liver (see page 326) but is appreciable in nuclei of rapidly growing tumor tissue or even in nuclei of rapidly growing normal tissue.

That the rapid accumulation of ^{32}P by nuclei of growing tissue may be attributed to mitotic activity is suggested by the following experiment carried out by Marshak.[154]
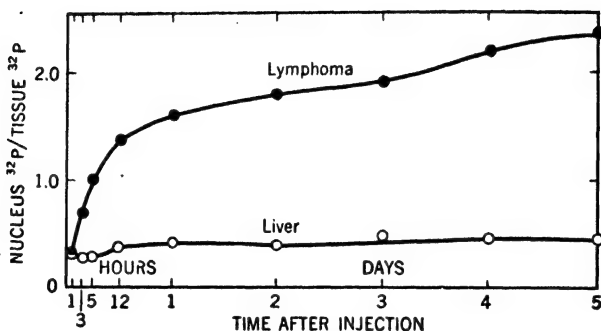
Fig. 66.   Nuclear activity as a fraction of tissue activity.[154]

The median and left lateral lobes of the livers of three 150-g. rats were removed, and labeled phosphate was injected by way of the femoral vein 36 hours later. Normal rats and rats bearing bilateral carcinoma 256 implants were injected at the same time.   Two days later the livers were cleared of blood by perfusion with saline, and the nuclei were isolated from the livers and tumors. For the normal liver the ratio of activity per milliliter of nuclei to activity per gram of tissue was 0.345, while for regenerating liver and tumor the ratios were 1.02 and 1.08, respectively.   When ^{32}P was injected 4 days after partial hepatectomy and the nuclei were isolated 3 days later, the ratios for normal and for hepatectomized animals were 0.28 and 0.32.   At this time very few nuclei were found in mitosis, while 36 hours after partial hepatectomy 3.7% were in anaphase or metaphase and many more were in prophase.

Hevesy[154a,155] investigated the uptake of ^{32}P by the tissue and nuclei of liver and Jensen sarcoma of the rat.   In these investigations the method of separation of nuclei introduced by Dounce[156] was applied. The ratio of specific activity of liver tissue phosphorus and liver nucleus phosphorus found in the different experiments is shown in Table 138, showing that an appreciable part of the labeled phosphate finds its way from the cytoplasm into the nuclei.   Table 139 contains data on specific

154a G. Hevesy, *Arkiv Kemi Mineral. Geol.*, **A24**, No. 26 (1947).
155 G. Hevesy, *unpublished data.*
156 A. L. Dounce, *J. Biol. Chem.*, **151**, 221 (1943).

activities of total phosphorus and phosphatide phosphorus of the tissue and nuclei, and also on residual phosphorus (phosphorus remaining after treatment with trichloroacetic acid, ether–alcohol, and chloroform)

TABLE 138

Ratio of Specific Activity of Total Tissue Phosphorus and Total Nucleus Phosphorus in Liver of Rats

Time after administration of ^{32}P, hr.	Ratio of specific activity of total tissue P to that of total nucleus P
1 (Marshak)	3.7
2 (Hevesy)	7.2 (Jensen sarcoma 5.2)
3 (Marshak)	2.5
5 (Marshak)	2.5

TABLE 139

Rate of Renewal of Total, Phosphatide, and Residual Phosphorus in the Tissue and Nuclei of Liver and Jensen Sarcoma of the Rat[155]

Fraction	Ratio of specific activity of fraction P to inorganic P of the organ[a]
Liver	
Total tissue P	27.80
Total nucleus P	3.86
Tissue phosphatide P	15.31
Nucleus phosphatide P	9.94
Nucleus residual P	3.21
Sarcoma	
Total tissue P	18.80
Total nucleus P	3.59
Tissue phosphatide P	2.91
Nucleus phosphatide P	2.65
Nucleus residual P	3.16

[a] Time of experiment = 2 hours. Mean values for 36 rats.

of nuclei. As the separation of nuclei takes place in acid solution, part of the acid-soluble phosphorus present in the nuclei is removed during their isolation and thus the value obtained for total ^{32}P content of nuclei depends upon the procedure employed.

VI. Phosphorus Turnover in Leukemic Tissue

In their studies on phosphorus metabolism of leukemic tissue Tuttle, Erf, and Lawrence compared the distribution of labeled phosphorus in the acid-soluble, phosphatide, and nucleoprotein fractions of tissues
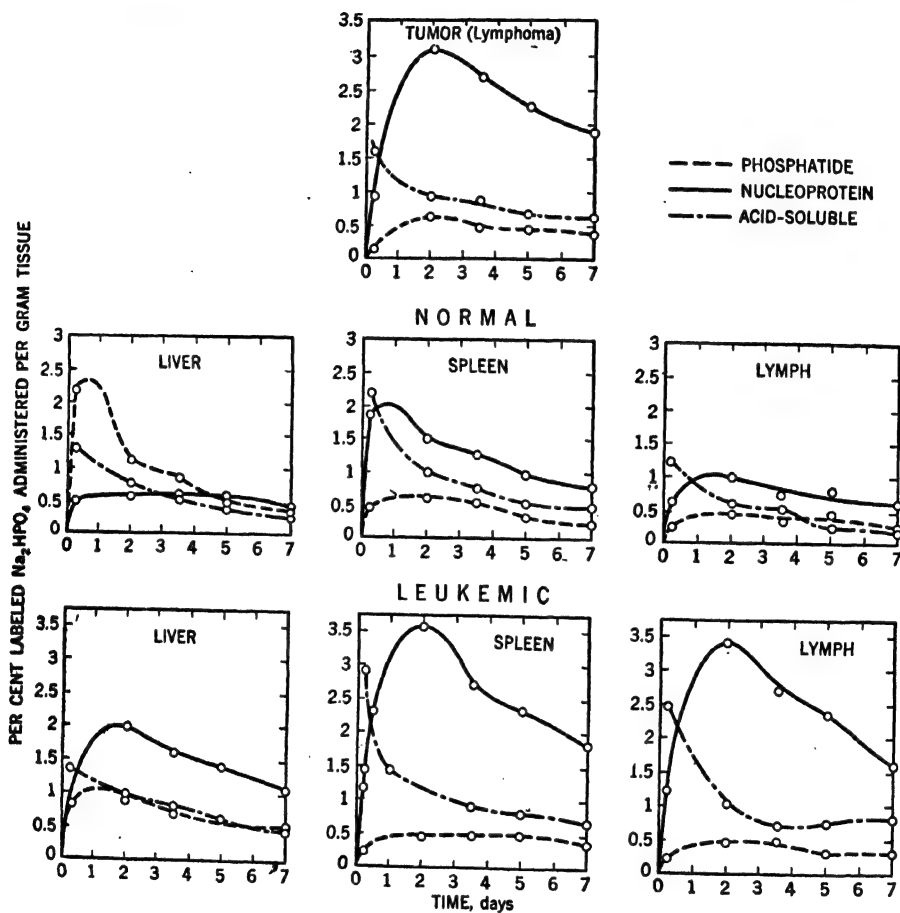
Fig. 67.   Comparison of the rates of formation and distribution of the different organic phosphorus fractions in tumor tissue and in normal and leukemic tissues.[157]

of normal and leukemic mice.[157]   Some of the results of these studies are shown in Figure 67.   The residual (nucleoprotein) fraction from tumor cells and from leukemic tissues contains considerably more radio-

[157] L. W. Tuttle, L. A. Erf, and J. H. Lawrence, *J. Clin. Invest.*, **20**, 57 (1941).

phosphorus than do normal structures. These results indicate that nucleoproteins are synthesized at a more rapid rate by leukemic and tumor cells than by normal tissue.

VII. Intravenous Transfer of ^{32}P from Chromatin to Hepatic Tissue

Marshak and Walker[158] injected labeled chromatin into the blood of rats weighing 50 g. after partial hepatectomy and found the liver nuclei retained much more ^{32}P than after injection of labeled inorganic phosphate of the same activity (see Table 140). The difference between

TABLE 140

^{32}P in Regenerating Livers Three Hours after Intravenous Injection of Labeled Substances[158]

Material injected	Number of rats	^{32}P/g. liver as per cent dose	Standard error of mean
Inorganic phosphate..................	84	4.52	0.083
Inorganic phosphate..................	10	4.36	0.084
Rat chromatin.........................	19	26.30	0.670
Lipide (rat chromatin)................	15	32.20	0.390
Fat-free chromatin (rat)...............	5	7.10	0.480
Adenosine triphosphate................	7	4.43	0.320
Rabbit chromatin......................	8	23.10	0.200
Rabbit chromatin soluble in 1 M NaCl...	8	16.20	0.470

^{32}P Uptake by Nuclei

Substance injected	Per cent dose/g. nuclei	Per cent liver ^{32}P in nuclei
Inorganic phosphate ....:....................	1.54	2.1
Rat chromatin.............................	5.08	1.2
Rabbit chromatin...........................	5.06	1.3
Rabbit chromatin soluble in 1 M NaCl.........	4.23	1.6
Fat-free chromatin (rat)....................	5.45	4.6
Lipide (rat chromatin)....................	5.15	1.0

uptake of ^{32}P by nuclei after administration of chromatin and inorganic phosphate is much less striking if the ^{32}P content of the nuclei is compared with the ^{32}P content of the liver tissue. This comparison seen in Table 140 shows that the liver tissue takes up a much larger percentage

[158] A. Marshak and A. C. Walker, *Am. J. Physiol.*, **143**, 235 (1944).

of the labeled chromatin than of the labeled phosphate. This is another example of the enhanced uptake of phosphorus compounds foreign to the plasma by the liver. If the labeled chromatin splits off inorganic labeled phosphate by enzyme action in the liver, we would expect enhanced formation of labeled compounds in the nuclei corresponding to the high ^{32}P level maintained in the liver.

That labeled desoxyribonucleic acid intravenously injected into rats weighing 100 – 200 g. rapidly splits off labeled phosphate in liver was shown by Ahlström and co-workers.[159] Some of their results are seen in Table 141. After the lapse of 2 hours an appreciable part of the labeled phosphorus is found in the liver, and more than three-fourths in the acid-soluble fraction.

TABLE 141

^{32}P of Injected Labeled Desoxyribonucleic Acid Taken up by Various Tissues[a] after Two Hours[159]

Rat	Plasma total P	Liver inorganic P	Liver acid-soluble P	Liver total P	Spleen total P	Kidney total P	Plasma inorganic P
I.........	2.2	11.0	14.5	18.0	0.79		0.81
II.........	8.8	5.6	7.4	9.4	0.48		0.34
III.........	1.7	8.2	10.6	14.3	0.92		0.57
VI.........	1.9	8.6	12.1	16.0	0.65		0.58
V.........	1.1	6.8	9.2	13.8	0.66	2.80	0.56
VI.........	1.0	3.7	5.5	8.7	0.38	1.46	0.30
VII.........	0.32	4.3	6.2	9.0	0.44	1.39	0.25
VIII.........	0.78	6.1	8.7	10.4	0.77	2.04	0.37
IX blood ... X blood....	3.5	10.2	13.4	20.7	0.85	4.7	
XI blood ... XII blood ...	2.2	9.5	11.9	18.8	0.75	3.7	

[a] Values are in per cent of injected ^{32}P.

In experiments in which liver slices were incubated in bicarbonate–Ringer solution containing labeled desoxyribonucleic acid, the phosphate group of more than two-thirds of the nucleic acid added was found to be split off in the course of 4 hours, four-fifths of the total acid-soluble ^{32}P being present in the phosphate fraction. The fact that, in contrast to

[159] L. Ahlström, H. v. Euler, G. Hevesy, and K. Zerahn, *Arkiv Kemi Mineral. Geol.*, **A22**, No. 7 (1946).

the phosphate group of desoxyribonucleic acid introduced into the circulation, phosphate of the desoxyribonucleic acid already present in the liver cells is not split off at an appreciable rate, may be due to the highly polymerized and shielded state in which nucleic acid exists in nuclei.[160]

Not only nucleic acid, but also other organic phosphorus compounds administered give off inorganic phosphate. Choline phosphate appears for example within a short time as inorganic phosphate in the circulating blood of the rat following intraperitoneal injection of the ester.[161] Triphenyl phosphite was found to be decomposed readily after being injected intraperitoneally.[162] Phosphatides which are normal constituents of plasma are not decomposed in the circulation at an appreciable rate.

While it is probable that most of the labeled phosphate in nuclei after intravenous injection of labeled chromatin was carried into the nuclei as free phosphate, the possibility of incorporation of some nucleoprotein into nuclei cannot be excluded. Griffith[163] and Avery and co-workers[164] have shown that pneumococci which, in artificial culture, have lost the capsules endowing them with virulence and containing the specific polysaccharides, revert to avirulent nonspecific types. Such degenerate, nonspecific pneumococci, from whatever specific type derived, could be induced, by cultivation in a medium prepared from a complete virulent type, to reacquire a capsule conferring the corresponding specificity.

Recently Avery and his colleagues were able to isolate and to characterize a chemical principle acting in minute dosage as the specific stimulus to such a transformation. The substance inducing the transformation was found to be a nucleic acid of the desoxyribose type. It is possible that this substance as such enters the nucleus and that we meet here one of the very few cases in which the organism avails itself of phosphorus compounds of high molecular weight of exogenous origin in fundamental synthetic processes.

[160] L. Ahlström, H. v. Euler, G. Hevesy, and K. Zerahn, *Arkiv Kemi Mineral. Geol.*, **A22**, No. 7 (1946).

[161] R. F. Riley, *J. Biol. Chem.*, **153**, 535 (1944); *J. Am. Chem. Soc.*, **66**, 512 (1944).

[162] R. B. Aird, W. E. Cohn, and S. Weiss, *Proc. Soc. Exptl. Biol. Med.*, **45**, 306 (1940).

[163] F. Griffith, *J. Hyg.*, **27**, 113 (1928).

[164] O. T. Avery, C. M. MacLeod, and M. McCarty, *J. Exptl. Med.*, **79**, 137 (1944).

VIII. Phosphorus Turnover in Yeast

When bakers' yeast cells (*Saccharomyces cerevisiae*) were suspended for 1.5 hours at 22°C. in (*a*) glucose–salt, (*b*) fluoride–glucose–salt, or (*c*) sustaining salt media, to each of which radiophosphorus in form of phosphate had been added, Lawrence et al.[165] found radioactivity only in those cells suspended in the first solution. The uptake of radiophosphorus varied with the concentration of glucose.

By use of ice-cold 5% trichloroacetic acid and hot ether–alcohol solutions, it was determined that approximately 80% of the activity was found in the acid-soluble and 20% in the nucleoprotein fractions. (Approximately 40% of the activity of the nucleoprotein fraction was present in the nucleic acids.) Iodoacetic acid decreased while cyanide increased the rate of uptake of radiophosphorus in the nucleoprotein fraction. Once the yeast cells had incorporated ^{32}P, it was not lost by resuspending the cells in a radiophosphorus-free medium. Hevesy and Zerahn[166] (see also Hevesy et al.[167]) found that yeast grown in a labeled nutrient solution, when kept at 15° for 1 day in a nonlabeled nutrient solution, lost about 1–3% of its ^{32}P content; about one-half of the ^{32}P lost by the yeast cells was found to be present in the nutrient solution as inorganic, and the other half as organic, acid-soluble phosphorus. Irradiation by ultraviolet rays, not however by an x-ray dose of 15,000 r., appreciably increased the loss of ^{32}P by the yeast cells.

It was furthermore found by Malm[168,168a] that uptake of ^{32}P by cells depends on the *p*H of the solution in which the cells are suspended. A maximum uptake takes place with *p*H values of 4–5, while at *p*H 7 practically no uptake of ^{32}P is observed. As *p*H of the cells is not influenced by changes in *p*H of the nutritive solution, this observation suggests that processes going on at the cell surface or within the cell boundary play an important part in phosphorus metabolism in yeast.

Lindahl and associates[169] found recently that radioactive phosphate introduced into the cells of bakers' yeast was incorporated into the coenzyme molecule. The rate of this process was under certain conditions dependent on the rate of metabolism; but the phosphate exchange

[165] J. H. Lawrence, L. A. Erf, and L. W. Tuttle, *J. Applied Phys.*, **12**, 333 (1941).
[166] G. Hevesy and K. Zerahn, *Acta Radiol.*, **27**, 157 (1946).
[167] G. Hevesy, K. Linderstrøm-Lang, and C. Olson, *Nature*, **140**, 725 (1937).
[168] M. Malm, *personal communication.*
[168a] M. Malm, *Arkiv Kemi Mineral. Geol.*, **A25**, No. 1 (1947).
[169] P. E. Lindahl, M. Malm, B. Strindberg, and B. M. Lagergren, *Nature*, **158**, 746 (1946).

also took place in the absence of exogenous substrate at low temperature ($+ 4°C.$),.though at a very slow rate. The observations of Spiegelman and Kamen on the release of residual ^{32}P by yeast is discussed on page 338.

IX. Studies of Virus Reproduction

Despite the fact that the introduction of one unit of a virus within a living cell of a susceptible host is followed by the production of millions of virus units, almost nothing is known about the reproductive process. It seemed possible that preparation and isolation of tobacco mosaic virus containing radioactive phosphorus and inoculation of this virus into the diseased plants with the subsequent extensive multiplication of the virus should provide some information concerning the process of virus reproduction. This line of thought induced Stanley[170] to investigate the radioactivity of Turkish tobacco plants inoculated with labeled tobacco virus. Similar investigations were also carried out by Born and associates.[171]

When mosaic-diseased Turkish tobacco plants were fed a nutrient solution containing radioactive phosphorus in the form of disodium phosphate over a period of several weeks, about 30% of the phosphorus taken up by the plants was isolated (by Stanley) in the form of purified tobacco mosaic virus. The tobacco mosaic virus growing, in contrast to the plant, at a rapid rate is bound to take up a large percentage of the labeled phosphate which reaches the plant. While an organ or a substance grown in a labeled medium becomes labeled throughout, parts of a mature organism became labeled only by interchange, the rate of which varies greatly with the organ and the compound in question.

The virus containing radioactive phosphorus, to a large extent present as a constituent of the ribonucleic acid molecule, was rubbed into the lower leaves of Turkish tobacco plants. After 12 days the lower inoculated and the upper uninoculated leaves were investigated, with the result shown in Table 142.

Since, in Stanley's experiments (as seen in Table 142), most of the radioactivity was found associated with nonvirus components in both inoculated and uninoculated portions of the plants, it was impossible to determine whether or not the small amount of radioactive virus

[170] W. M. Stanley, *J. Gen. Physiol.*, **25**, 881 (1942).
[171] H. J. Born, A. Lang, G. Schramm, and K. G. Zimmer, *Naturwissenschaften*, **29**, 222 (1941).

found in the uninoculated portions resulted from movement of the inoculated virus. In view of these results it is exceedingly difficult to

TABLE 142

Distribution of ^{32}P in Turkish Tobacco Plants Twelve Days after Inoculation of Lower Leaves with 58 Mg. Labeled Tobacco Mosaic Virus[170]

Fraction	Relative activity
Virus isolated from inoculated leaves..............	8.3
All material of inoculated leaves except virus........	33.4
Virus isolated from uninoculated leaves............	5.8
All material of uninoculated leaves except virus	52.5

distinguish between ^{32}P taken up by the plant in the form of virus and that taken up in the form of virus disintegration products.

X. Dynamic State of Body Constituents

Perhaps the most remarkable result obtained in the study of the application of labeled phosphorus and of other radioactive and stable (see Schoenheimer[172]) indicators is the discovery of the dynamic state of the body constituents. The molecules constituting the plant or animal organism are incessantly renewed. In the course of this renewal, not only the atoms and molecules taken up with the food participate, but atoms and molecules located in one organ or in one type of molecule will soon be found in another organ or in another type of molecule present in the same or in another organ. A phosphate radical taken up with the food may first participate in the phosphorylation of glucose in the intestinal mucosa, soon afterward pass into the circulation as inorganic phosphate, enter a red corpuscle, become incorporated with an adenosinetriphosphoric acid molecule, participate in a glycolytic process going on in the corpuscle, return to the circulation, penetrate the liver cells, participate in the formation of a phosphatide molecule, and after a short interval enter the circulation in this form, penetrate marrow, and leave this organ after some time as a constituent of a lymphocyte. We may meet the phosphate radical again as a constituent of the plasma, from which it may find its way into the skeleton. Being incorporated in the uppermost molecular layer of the skeleton, it

[172] R. Schoenheimer, *The Dynamic State of Body Constituents.* Harvard Univ. Press, Cambridge, 1942.

will have a good chance of being replaced by other phosphate radicals of the plasma or the lymph, but it may also have the good fortune to find a more or less lasting abode in the skeleton. This will be the case when it becomes embedded in a newly formed apatite-like bone crystallite (*cf.* Hevesy[5b]).

There are indications that, in the growing organism, the rate of new formation of molecules is appreciably greater than in a fully grown organism. It was found, for example, that, in the liver of 4-day-old rats besides an appreciable formation of additional desoxyribonucleic acid, a very appreciable renewal of "old" molecules takes place as well. The turnover of desoxyribonucleic acid in the liver of 4-day-old rats is about 20 times as rapid as the corresponding value found in fully grown rats.[173]

XI. Turnover of Sulfur Compounds

The fate of sulfur compounds in the organism has not yet been investigated so extensively as that of phosphorus compounds. This is due to various factors. Radiosulfur was formerly more difficult to obtain than radiophosphorus. The radiation emitted by radiosulfur being very soft (*cf.* page 47), its measurement is less convenient than that of ^{32}P. Furthermore, while we can study the turnover of phosphorus compounds by administering labeled phosphate, which becomes incorporated in the various phosphorus compounds present in the organism, we have to administer methionine, cystine, or similar compounds for studying the turnover of sulfur.[173a-175] The animal organism cannot avail itself of administered sulfate in building organic compounds containing sulfur[174] (see also Fromageot[176]).

A. FATE OF ELEMENTARY SULFUR FED TO RATS

In the experiments of Tarver and Schmidt[174] (see also these authors[177]) normal adult rats are fed with 1 mg. of labeled colloidal sulfur by stomach tube. After the lapse of 48 hours less than 0.3% of the

[173] L. Ahlström, H. v. Euler, and G. Hevesy, *Arkiv Kemi Mineral. Geol.*, **A19**, No. 9 (1944).

[173a] J. L. Tuck, *J. Chem. Soc.*, **1939**, 1292.

[174] H. Tarver and C. L. A. Schmidt, *J. Biol. Chem.*, **130**, 67 (1939); **146**, 69 (1942).

[175] R. G. Franklin, *Science*, **89**, 298 (1939).

[176] C. Fromageot, *Advances in Enzymology*, Vol. VII.   Interscience, New York, 1947, p. 369.

[177] H. Tarver and C. L. A. Schmidt, *J. Applied Phys.*, **12**, 323 (1941).

radiosulfur can be detected in the proteins of the liver or other internal organs. About 60% is recovered in the urine and 8% in the feces. When, however, 1 mg. labeled methionine is administered to fasting rats, 56% is found in the proteins (14% in the liver) and 36% is oxidized to sulfate.

B. FATE OF SULFUR FED AS SODIUM SULFIDE TO RATS

When labeled sulfur (11.10^6 counts per minute) was administered as sodium sulfide (containing 1.66 mg. sulfur) by stomach tube to rats, a very large part of the sulfur was absorbed and excreted in the urine. The major portion of the radioactive sulfur in the urine was in the form of

TABLE 143

Concentration of ^{35}S in Excreta and Tissues of Rats 24 Hours after Oral Administration of ^{35}S as Sodium Sulfide[178]

Tissue	Activity of tissue S (counts $\times 10^{-4}$ per mg. S per min.)	
	Rat I	Rat II
Urine		
Inorganic S	50.94	45.70
Total sulfate S	54.85	53.85
Total S	73.08	52.45
Feces	23.71	7.52
Gastrointestinal tract	5.13	5.34
Bones	1.43	1.33
Heart	0.82	0.58
Kidneys	0.52	0.48
Spleen	0.42	0.34
Gonads	0.26	0.38
Lungs	0.34	. . . . .
Blood	0.21	0.25
Liver	0.17	0.28
Brain	0.02	0.17
Skin	0.53	0.16
Muscle	0.046	0.036
Hair	0.0003	0.0008

sulfate sulfur, both inorganic and ethereal.[178] This result indicates that the rat can oxidize sulfide sulfur to sulfate sulfur and thereby change a relatively toxic form of sulfur to an innocuous form. The concentration

[178] D. D. Dziewiatkowski, *J. Biol. Chem.*, **161**, 723 (1945).

of labeled sulfur in excreta and tissues of rats 24 hours after oral admin-
istration is seen in Table 143. Dziewiatkowski[179] succeeded also in
showing that some of the radioactive sulfur administered as sulfide is
incorporated in the mercapturic acid synthesized after bromobenzene
administration and is also present in the cystine isolated from hair,
liver, skeletal muscle, and skin. The amount of cystine synthesized
by the process employing sulfide sulfur was found to be very small
(see Table 144), even though there was an active deposition of protein
as indicated by an increase in body weight.

TABLE 144

Activity of Cystine Sulfur Isolated from Rat Tissue after Intraperitoneal
Injection of Sodium Sulfide Containing Radioactive Sulfur[a]

	Counts per minute per milligram S			
Cystine source	Rat A	Rat B	Rat C	Rat D
Hair.............	50	86	3	4
Skin.............	22	17	5	25
Muscle...........	61	166	4	13
Liver.............	16	17	2	8

[a] A total of 11 mg. sulfur and 10.9×10^5 counts in the course of three days.

Singher and Marinelli[180] administered labeled heptaldehyde bisul-
fite, cinnamaldehyde bisulfite, and sodium sulfate intraperitoneally to
rats fasted for 14 to 16 hours, at the end of which they were sacrificed.
The highest concentration of radioactive sulfur was found to occur in
the bone marrow irrespective of the sulfur compound used. The
relative distribution of sulfur in hair, brain, and some other compounds
was found to depend upon the chemical structure of the compound.

C. RATE OF REPLACEMENT OF PROTEIN SULFUR

Labeled methionine was administered in the studies of Tarver and
Schmidt.[174] One day after administration of methionine the replace-
ment figures shown in Table 145 were found in proteins isolated from the
tissues of fasting fistula dogs. The most rapid replacement was found[174]
to occur in intestinal mucosa and pancreas, the slowest in leg muscles
and red corpuscles. How far these low replacement figures are due to a

[179] D. D. Dziewiatkowski, *J. Biol. Chem.*, **164**, 165 (1946).
[180] H. O. Singher and L. D. Marinelli, *Science*, **101**, 414 (1945).

low rate of replacement of the proteins or a low rate of penetration of methionine or an intermediary protein sulfur precursor is not known.

In the protein of another dog killed after the lapse of 30 hours, the sulfur replacement percentage in fibrin was found to be 0.15, in pseudoglobulin and albumin 0.14 and 0.16, respectively. As for liver proteins, the replacement percentage was found to be 0.16. The methionine administered has about the same chance to be present, after the lapse of 30 hours, in liver, fibrinogen, pseudoglobulin, and albumin proteins.

TABLE 145

Extent of Replacement of Protein Sulfur One Day after Administration of Labeled Methionine[174]

Organ	Per cent replacement protein S by labeled S	Organ	Per cent replacement protein S by labeled S
Liver	0.41	Pancreas	0.47
Kidney	0.30	Leg muscles	0.01
Spleen	0.18	Red corpuscles	0.01
Lungs	0.17	Stomach mucosa	0.24
Brain	0.08	Intestinal mucosa	0.81
Thyroid gland	0.15		

The sulfur present in different liver and plasma protein fractions (pseudoglobulin, euglobulin, albumin 1, and albumin 2) has the same specific activity, while the specific activity of cystine sulfur is found to be only two-thirds of the specific activity of the residual methionine sulfur. From this result it is apparent that the conversion of methionine sulfur to cystine sulfur is not a slow process. Radioactive cystine is found in liver and kidney proteins.

In later experiments, Tarver et al.,[181] investigating methionine turnover in hepatectomized dogs, found that when labeled methionine in small doses (1 mg. per kg.) is injected into dogs, 97% or more of the dose is retained in the animal. In a 4-day period, 12% or less is excreted in the urine. In a period of 2 to 5 hours, considerable amounts of methionine sulfur are incorporated into the protein in tissues such as kidney, pancreas, and intestinal mucosa of both the control sham-operated animals and the animals with livers completely removed. The result obtained indicates that, in the animals without a liver, as much

[181] H. Tarver and W. O. Reinhardt, *J. Biol. Chem.*, **167**, 395 (1947).

or more of the labeled amino acid is incorporated into their proteins as in the animals with a liver. Thus the liver is not essential for synthesis of protein in other tissues. The results permit the conclusion that the liverless animal retains a significant fraction (one-seventh) of the control animal's capacity to synthesize globulin. The rate of albumin synthesis in the normal dog is 20 or more times that found in the hepatectomized animal.

When labeled methionine is injected into the circulation of the rat, the specific activity of the plasma methionine reaches its maximum after the lapse of 6 hours; that of the intestinal mucosa after 16 hours. The maximum specific activity of the intestinal mucosa is about twice that of the plasma. The specific activity values of the liver after the lapse of 24 hours is almost the same as the plasma value. The red corpuscle values increase all through the experiment taking 7 days.[174]

The introduction of radiosulfur into proteins after radioactive methionine feeding indicates a synthesis of peptide bonds. That the opening and reclosing of peptide bonds is a rapid process is shown by numerous investigations by Schoenheimer and co-workers,[172] and the above results of Tarver and Schmidt[174] can be interpreted as a further proof of the rapid turnover of tissue proteins. Du Vigneaud and associates[182] applied the stable sulfur isotope ^{34}S as a tracer. When administering labeled methionine to rats kept on a diet free of cystine, up to 80% of the derived cystine produced in the rats was found to contain sulfur from methionine.

When labeled pseudoglobulin or labeled albumin is added to defibrinated dog plasma, no significant transfer of radiosulfur is found to take place from either the pseudoglobulin or the albumin to the other proteins in the plasma, though the data do not exclude the possibility of some exchange between euglobulin and pseudoglobulin.

Experiments undertaken to determine whether or not methionine sulfur can be converted to taurine sulfur by the dog and the rat tended to show positive results. Taurine containing radiosulfur was isolated from the bile of fistula dogs given radioactive methionine. A large part of the labeled sulfur (as methionine and cystine) is contained in the proteins of fasted bile fistula dogs and rats fed methionine.

An enzyme system, present mainly in liver, forms hydrogen sulfide, pyruvic acid, and ammonia from cystine. The same enzyme system was

[182] V. du Vigneaud, G. L. Kilmer, J. R. Rachele, and M. Cohn, *J. Biol. Chem.*, **155**, 645 (1944).

found to form hydrogen sulfide containing radiosulfur and, to a less extent, active cystine from inactive cystine. When 76 mg. inactive cystine and 1.53 mg. active hydrogen sulfide were added to the enzyme system by Smythe and Halliday,[183] 7.5 mg. cystine was recovered and contained 0.7% of the activity.

The rate of appearance of ^{35}S in the proteins of egg white was determined by feeding methionine-containing radioactive sulfur to hens. [35]S was first found in the egg white on the second day and reached its maximal value on the fourth.[177]

D. FATE OF PLASMA PROTEINS TAGGED WITH RADIOACTIVE SULFUR

Labeled plasma was obtained by Seligman and Fine[184] on feeding cystine, homocystine, or methionine-containing radiosulfur, the highest concentration of amino acids in the plasma protein being obtained with cystine. The largest percentage utilization of amino acid in the production of radioactive plasma protein is obtained with cystine as well. In Table 146 data are given for the percentage incorporation of radioactive amino acids into plasma protein.

TABLE 146

Incorporation of Radiosulfur in Plasma Proteins of the Dog[184]

Amino acid	Weight fed, mg.	Highest concn. of amino acid in protein fraction of plasma, mg./ml.	Ingested amino acid	
			Incorporated in plasma proteins, %	Excreted in urine as sulfate, %
l-Cystine........	100	0.024	5.2	36
l-Cystine........	200	0.046	14.8	19
dl-Homocystine...	500	0.0032	0.15	66
dl-Homocystine...	50[a]	0.0031	0.9	7
dl-Methionine....	50	0.00034	0.06	18
dl-Methionine....	150	0.0000	0.00	14

[a] Injected intravenously.

Application of labeled plasma proteins to the study of traumatic shock is made possible by the fact that the labeled proteins disappear from plasma at a low rate (Figure 68). For plasma protein containing radioactive sulfur, 90% of the radioactive protein was circulating 5

[183] C. V. Smythe and D. Halliday, *Federation Proc.*, **1**, 134 (1942).
[184] A. M. Seligman and J. Fine, *J. Clin. Invest.*, **22**, 265 (1943).

hours after injection, 70% 15 hours after injection, and 45% 48 hours after injection. Figure 68 shows the rate of disappearance of radioactive sulfoproteins and radioactive bromoproteins of the circulating plasma of dogs. Radiobromoprotein containing less than 0.1% bromine disappears at a somewhat slower rate from the plasma than does radioactive sulfoprotein. The synthetic methods developed by du Vigneaud and co-workers and by Tarver and Schmidt were utilized in preparing from barium sulfate amino acids containing radioactive sulfur. Cystine was obtained in 17% yield and homocystine in 25% yield.[185]
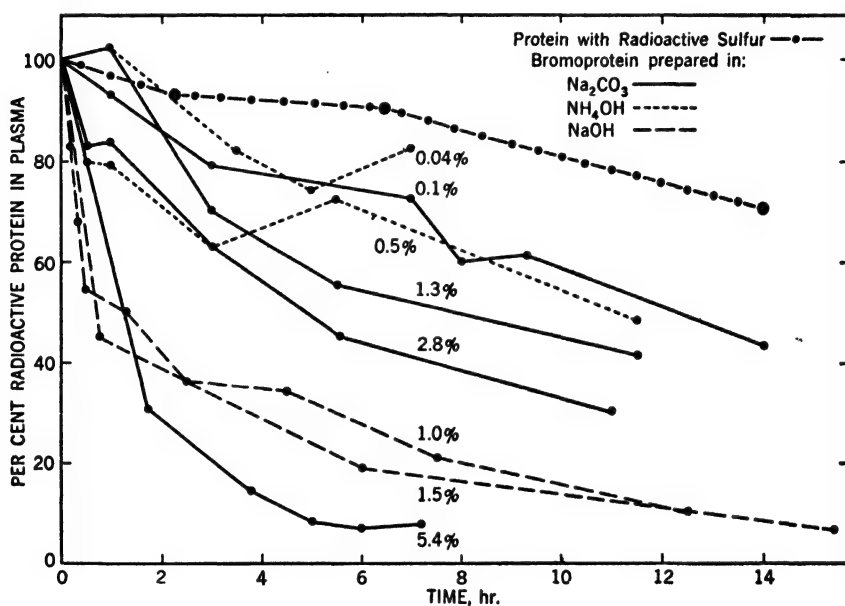
Fig. 68. Rate of disappearance of radioactive sulfoprotein and bromo-protein (containing various percentages of bromine and prepared in several ways) from the circulating plasma of normal anesthetized dogs, compared with radioactive plasma protein containing radioactive sulfur.[186]

Labeled proteins were also applied in the study of the loss of plasma proteins in hemorrhagic shock.[186] A prevailing opinion is that increased permeability of the capillaries exists in shock and that a consequent loss of plasma into tissues ensues such that the effective circulating blood volume falls to a level incompatible with life. This loss of plasma

[185] A. M. Seligman, A. M. Rutenburg, and H. Banks, *J. Clin. Invest.*, **22**, 275 (1943).
[186] J. Fine and A. M. Seligman, *J. Clin. Invest.*, **22**, 285 (1943).

is presumed to occur generally throughout the body as well as in areas of local injury, because replacement of all the plasma or of more than can be accounted for as lost at the site of injury does not sustain the
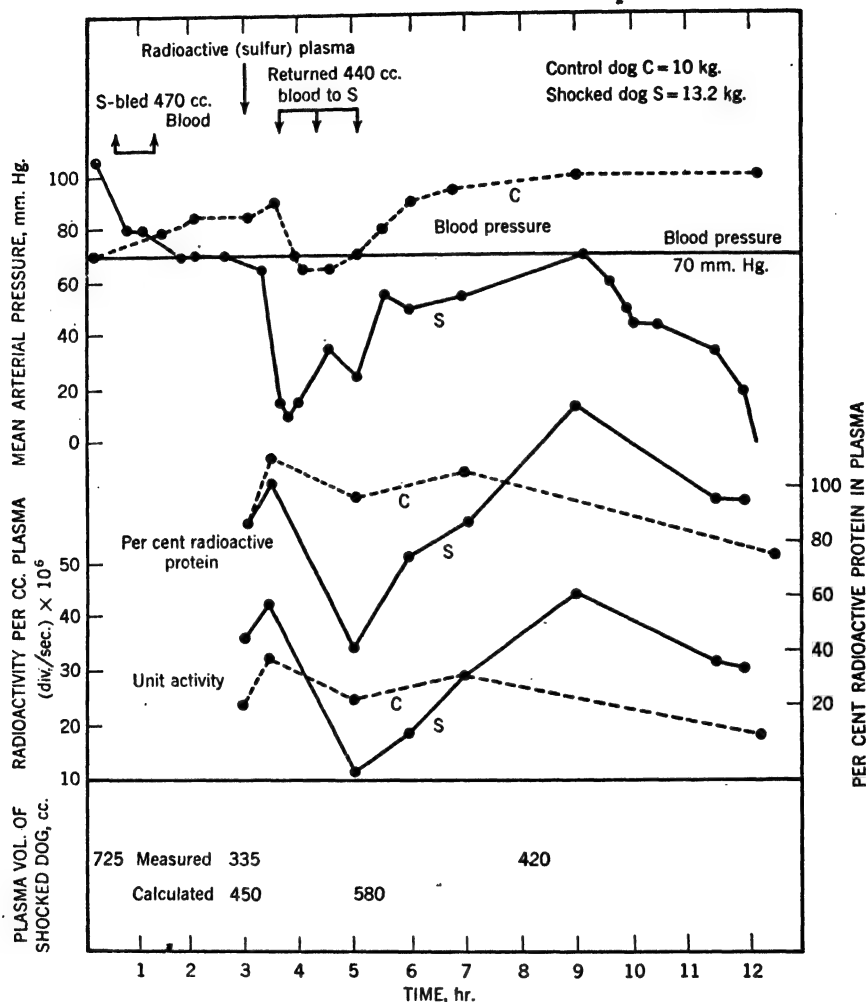
Fig. 68A. Rate of disappearance of labeled proteins from the plasma of normal and of shocked dogs.

organism.   When radioactive proteins were given intravenously to normal dogs and dogs in hemorrhagic shock, both with and without anesthesia, Fine and Seligman[186] (see also Seligman, Rutenberg, and Banks[185]) found no difference in the disappearance curves of the radio-

active protein. Tissue analysis of radioactive protein in these dogs gave the same result. However, when the shocked dogs received intravenous infusion in the late or irreversible phase of shock, the radioactivity content of some tissues showed that plasma protein was lost into these tissues as seen in Figure 68A in possibly significant quantity.

It is concluded from the experiments described that, while the integrity of capillaries may be impaired in the late shock phase, there is no evidence of significant loss of plasma into tissues in untreated fatal shock following hemorrhage. Hence, an increase in capillary permeability is not a factor in the fatal issue.

Similar experiments were carried out with plasma produced by administering to dogs the amino acid lysine synthesized with heavy nitrogen: 50% of the labeled protein was found to have left the blood stream in about 24 hours; 75% in about 6 days. Shock due to trauma of intestine or leg was found to show a dilution curve of labeled protein not unlike that of the normal dog.[187]

E. UPTAKE OF LABELED SULFUR BY PROTEINS OF LIVER SLICES

When surviving liver slices (0.7 to 1.9 g.) of the rat liver were incubated in 5 ml. Krebs saline with 0.2% glucose containing labeled methionine varying over a range of 4.2 to 42 micromoles, about 0.5 micromole of methionine sulfur was found to be taken up by per gram liver slice.[188] The data obtained are shown in Table 147. The slices contained about 50 micromoles methionine; thus about 1% of the methionine sulfur was replaced during the experiment taking 1.5 to 2 hours. This is the result assuming that all labeled sulfur is introduced as methionine, which is not strictly the case. The percentage uptake of ^{35}S by the slices decreases, as is seen in the table when the concentration of methionine in the Krebs saline is increased.

Table 148 demonstrates that homogenization of the tissue reduces the uptake of methionine to a very low value. This observation indicates that the uptake of methionine by the slices is an enzymic process depending on the preservation of the enzyme system. A very different result was found[189] when studying the uptake of cystine by tissue. Both

[187] R. M. Fink, T. Enns, C. P. Kimball, H. E. Silberstein, W. F. Ball, S. C. Madden, and G. H. Whipple, *J. Exptl. Med.*, **80**, 455 (1944).

[188] H. Tarver, *personal communication.*

[189] H. Tarver and C. L. A. Schmidt, *J. Biol. Chem.*, **167**, 387 (1947).

the incubation of labeled cystine with slices and with homogenates results in an uptake of ^{35}S by the proteins. The major part of the uptake is in this case due to the formation of disulfide bonds, as shown by reduction studies.

TABLE 147

Incubation of Methionine with Rat Liver Slices[188]

| Rat No. | Tissue wet wt., g. | Incubation time, hr. | Counts | Substrate, μM | Per cent labeled sulfur found as | | | | Total ^{35}S recovered, % |
					Protein	Cystine	Sulfate	Methionine	
1	1.33	1.5	50,000	38	1.1	11.6	3.4	—	—
1	1.97	1.5	50,000	38	1.3	12.4	4.2	—	—
2	0.68	1.5	22,000	21	1.3	——	3.8	—	—
2	0.71	1.5	22,000	21	1.2	13.5	4.1	84	103
2	0.83	1.5	22,000	210	0.51	14.6	0.8	80	96
3	0.55	1.5	22,000	21	0.58	16.2	4.4	85	106
3	0.58	1.5	22,000	84	0.45	17.4	0.9	69	88
3	0.69	1.5	22,000	210	0.37	10.8	0.6	90	102
4	0.75	1.5	22,000	21	1.4	17.2	5.0	—	—
4	0.96	1.5	22,000	21	1.7	21.4	5.8	—	—
4	0.90	1.5	22,000	84	1.1	20.0	2.2	72	95

TABLE 148

Effect of Homogenizing Tissue on Uptake of Labeled Methionine[188]

| Tissue wet wt., g. | Substrate methionine, micromoles | Labeled sulfur found, micromoles | | |
		Protein	Cystine	Sulfate
1.4 (slices)............	12	0.5	—	2.8
1.1 (homogenate)......	12	0.05	—	0.1
1.1 (homogenate)......	12	0.10	—	0.2
2.4 (slices)............	5	0.2	0.8	0.8
1.7 (homogenate)......	5	0.005	0.08	0.12
1.5 (homogenate)......	5	0.003	0.06	0.10
(slices)[a]...........	7	0.06	0.3	0.2
(homogenate)[a].....	7	0.002	0.3	0.04

[a] These preparations were incubated 1 hour in 6 ml. total volume. Slices and homogenate were in comparable amounts.

F. THIAMIN METABOLISM IN MAN

Thiamin synthesized from radiosulfur was injected intramuscularly in two series of experiments, using a human subject on a normal and on a thiamin-free diet. Determinations of the free (unphosphorylated)

thiamin in the urine were made by the thiochrome method, and the radioactive sulfur of the feces and of the inorganic sulfur, ethereal sulfur, and neutral sulfur compounds in the urine were determined.

Rapid destruction of the injected thiamin was indicated in both experiments by the appearance of the radiosulfur in the inorganic fraction of the urine, in amounts increasing to about 15% of that injected daily. No radiosulfur was found in the ethereal fraction. In the neutral sulfur fraction of the urine, the excreted radiosulfur during the period of injections was less than that corresponding to the free thiamin found, indicating rapid interaction of the injected material with that already present in the tissues, and the displacement of pre-existing thiamin. After 36 days on the thiamin-free diet, injection of 8 mg. radiothiamin over a period of 3 days resulted in excretion of 0.8 mg. pre-existing thiamin. On discontinuing the injections, destruction of thiamin was again indicated by appearance of quantities of radiosulfur greater than that corresponding to free thiamin.

On the normal diet a total of 61% of injected thiamin was recovered from the urine and 11% from the feces over the period of the experiments. Of the urinary radiosulfur recovered, 25% represented destroyed thiamin appearing as inorganic sulfate and 18% destroyed thiamin appearing with neutral sulfur compounds.[190]

G. FATE OF MUSTARD GAS, THE SULFONE, AND THE SULFOXIDE IN THE ANIMAL BODY

Experiments were performed by Boursnell et al.[191] to determine the fate of mustard gas, bis(2-chlorodiethyl) sulfide, and some similar substances injected intravenously into rabbits, to study the distribution of the substance, or products of its metabolism in tissues, and its excretion from the animal body. In this work mustard gas containing radioactive S was used, and determinations were made of the radioactive S content of the blood, tissues, urine, and bile at intervals after the injection of 5 mg. labeled sulfur per kilogram body weight. The injected material rapidly diffused from the blood stream, and appreciable amounts of radiosulfur-containing compounds appeared in the urine and bile less than 20 minutes after the injection.

There was widespread distribution of radiosulfur in the tissues of

[190] H. Borsook, J. B. Hatcher, and D. M. Yost, J. Applied Phys., 12, 325 (1941).

[191] J. C. Boursnell, J. A. Cohen, M. Dixon, G. E. Francis, G. D. Grenville, D. M. Needham, and A. Wormall, Biochem. J., 40, 756 (1946).

the injected rabbits. Kidney, lung, and liver gave the highest values of all tissues examined, and it is suggested that the high values for these organs are due to their excretory functions. The greater part, and sometimes all, of the tissue radiosulfur in these injected rabbits was not extractable by ethanol, acetone, or ether, and it is believed that at least part of this "fixed" radiosulfur was present in protein complexes. Appreciable amounts of radiosulfur were excreted in the urine and bile of the injected rabbits; it is suggested that the latter mode of excretion might account for at least part of the intestinal damage occurring in mustard gas poisoning. No evidence has been obtained of preferential retention of the injected mustard gas, or of its radiosulfur-containing derivatives, in the fatty tissues of the body, or in any organ, with the exception of kidney, lung, and liver.

When labeled β, β'-dichlorodiethyl or -sulfone, which are oxidation products of mustard gas, was injected intravenously,[192] this substance was distributed fairly rapidly through the body, and appreciable amounts of radioactive sulfur were found in all tissues that were examined 1 hour after the injections. The injected rabbits also excreted appreciable quantities of radiosulfur in the urine and/or bile. A comparison of the results of experiments with labeled mustard gas, labeled sulfoxide, and labeled sulfone suggests that the three substances are similarly distributed throughout the body after injection, and that they all react with the tissues to form acetone- and ether-insoluble radiosulfur-containing compounds ("fixed" radiosulfur).

Mustard gas reacts under physiological conditions (pH 7.5 to 8.0 and at 37°) with many widely differing proteins to form compounds from which the mustard gas cannot be removed by long extraction with ethanol, acetone, etc. The conditions governing this reaction and the amount of mustard gas combining with several proteins have been studied with the aid of mustard gas containing radioactive sulfur (see Banks and co-workers[193]). The observations that mustard gas readily reacts with serum proteins, nucleoprotein, keratin, and collagen have been fully confirmed. The maximum amount of mustard gas which combines with human serum proteins is about 23 millimoles per 16 g. protein nitrogen, equivalent to 23 moles mustard gas per mole protein (assuming a mean molecular weight of 100,000 for serum proteins). This

[192] J. C. Boursnell, G. E. Francis, and A. Wormall, *Biochem. J.*, **40**, 765 (1946),
[193] T. E. Banks, J. C. Boursnell, G. E. Francis, F. L. Hopwood, and A. Wormall. *Biochem. J.*, **40**, 745 (1946).

amount is sufficient to account for the immunological specificity of mustard-gas-treated proteins.   In the experiments with nucleoprotein and serum albumin the ^{35}S contents of the mustard-gas-treated proteins corresponded to 36 and 34 millimoles mustard gas per 16 grams protein nitrogen, respectively, but there is no reason to believe that even these values represent the maximum combining capacities of these proteins for mustard gas.   Mustard gas reacts more readily with serum proteins than it does with gelatin under comparable conditions.   The action of mustard gas on certain proteins leads to a reduction of the iodine-titratable sulfhydryl of the protein, but this accounts for only a small part of the reaction.   Other experiments have indicated that there is no extensive reaction between mustard gas and the free amino groups of proteins.

The immunological properties of proteins treated with mustard gas and its sulfone were studied also by Wormall et al.[194]   They carried out serological inhibition tests with amino acid compounds of mustard gas and the sulfone, in order to obtain information about the immunologically dominant groups in the protein antigens of mustard gas and of the sulfone. Complete inhibition of the precipitin reaction between the sulfone–proteins and antisera to the sulfone–proteins was effected by "sulfone-glycine" (I) or by "sulfone-alanine" and there was partial inhibition with a mustard gas–glycine compound (II).

$$(I)\ O_2S \underset{CH_2\cdot CH_2}{\overset{CH_2\cdot CH_2}{<\ \ \ >}} N\cdot CH_2\cdot COOH \qquad S \underset{CH_2\cdot CH_2}{\overset{CH_2\cdot CH_2}{<\ \ \ >}} N\cdot CH_2\cdot COOH\ (II)$$
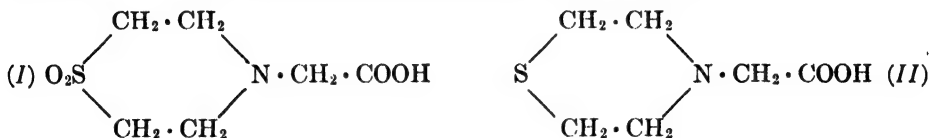
This suggests that the action of mustard gas–sulfone on proteins is largely concerned with the free amino groups of the protein:

$$RNH_2 + O_2S(CH_2\cdot CH_2Cl)_2 \rightarrow RN(CH_2\cdot CH_2)_2SO_2 + 2\,HCl$$
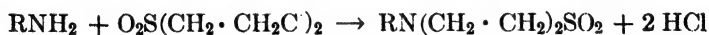
The precipitin reaction between mustard gas–proteins and their antisera is only slightly inhibited by II and in this and other respects there is a marked difference between the action of mustard gas and that of the sulfone on proteins.   The action of mustard gas and some related compounds on complement was also studied.[194a]

The distribution of two war gases, mustard and lewisite, labeled with

[194] J. C. Boursnell, G. E. Francis, and A. Wormall, *Biochem. J.*, **40**, 768 (1946).
[194a] J. C. Boursnell, G. E. Francis, and A. Wormall, *Biochem. J.*, **40**, 774 (1946).

radioactive sulfur (^{35}S) and radioactive arsenic (^{74}As), respectively, in skin and eye tissues has been studied using Axelrod's radioautographic technic[194b] by Axelrod and Hamilton.[194c]

In human skin, lewisite was found to be fixed primarily in the epidermis, with very small amounts found in the dermis. The lewisite present in the dermis was found in some blood vessels, in regions of the perivascular exudate, in some hair follicles, and in one case in a sebaceous gland. There was massive necrosis of most of the epidermal layer and corium resulting from the lewisite.

Comparable studies with mustard gas applied on human skin showed this material to be fixed in epidermis and dermis. The blackening of the autographs was so great, due to the accumulation of mustard gas, that it was impossible to determine the specific concentration by blood vessels; hair follicles were seen so rarely that it was difficult to determine whether or not they fixed mustard. The marked degree of necrosis noted with lewisite gas was not apparent with the mustard gas; this may be explained by the fact that injury by mustard gas is not detectable within 24 hours, whereas the effect of lewisite is more rapid and necrotic effects are visible within a much shorter time.

A long exposure of pig skin to mustard gas (6 hours to 2 mg.) showed a high concentration in epidermis, dermis, hair follicles and adjacent sebaceous glands, and blood vessels. A small amount was found in the hypodermis in the bands of fibrous tissue surrounding fatty tissue, and also in deep blood vessels.

Shorter exposures of pig skin to mustard gas (15 minutes to 475 g.) showed concentration in epidermis, dermis, and hair follicles.

Short exposures of pig skin to lewisite (15 minutes to 1.269 mg., and 1 hour to 1.534 mg.) showed concentration primarily in hair, superficially located hair follicles, and a very small amount in epidermis.

In all of the autographs, mustard gas penetrated the skin much more deeply than lewisite with a corresponding exposure. This deep penetration could explain the great destruction and deep burns resulting from exposure to mustard gas. In both the mustard and lewisite studies an accumulation of these two materials was noted in and around blood vessels. With destruction of blood vessels and subsequent local anemia one would expect slow healing of the affected skin area to ensue, which is a characteristic feature of these burns.

[194b] D. J. Axelrod, *Anat. Record*, **98**, 19 (1947).
[194c] D. J. Axelrod and J. G. Hamilton, *Am. J. Path.*, **23**, 389 (1947).

In rabbit eyes exposed to mustard gas this material was fixed primarily in the cornea, with a small amount in the conjunctiva, and a very small amount in the iris and lens.

XII. Role of Iodine in Thyroid Metabolism Turnover of Diiodotyrosine and Thyroxine

A. FORMATION OF LABELED DIIODOTYROSINE AND THYROXINE

The introduction of radioiodine as an indicator greatly enlarges our knowledge of the relation of the thyrotropic hormone to iodine metabolism and thyroxine synthesis by the thyroid gland.

Labeled thyroxine is rapidly formed in the thyroid gland. As early as 2 hours after administration of a tracer dose of ^{131}I to rats,[195] 1.5 to 3% of it was found by Chaikoff and his associates to be retained as thyroxine in the entire thyroid glands, which weighed $11 - 21$ mg. Increased amounts were found at a later stage. Table 149 records the distribution of the total radioiodine taken up by the thyroid gland of the rat among the thyroxine, diiodotyrosine, and inorganic fractions (*cf.* also Leblond *et al.*[195a]). Similar results were obtained in experiments with sheep. A larger percentage of administered ^{131}I was found as diiodotyrosine than as thyroxine at all stages. Despite a good deal of fluctuation in the actual amounts of labeled iodine deposited as thyroxine and diiodotyrosine in the gland, the proportion of the total labeled iodine represented by each of the fractions remained fairly constant at each time interval. These results suggest that diiodotyrosine is a precursor of thyroxine. Leblond *et al.*[196] found that iodine after entering the thyroid gland as inorganic iodine was rapidly transformed into diiodotyrosine and deposited as such in the colloid of the thyroid follicle.

Exposure of rats to cold $(0 - 2°C.)$ for various periods produces thyroid stimulation which is definite after 7 days, reaches a maximum after 26 days, but disappears after exposure for 40 days.[196] At the time of maximum stimulation by cold, the fixation of ^{132}I by the gland is 2.7 times that in the controls. Separation of the iodine fractions of thyroid at various times indicates that the turnover of thyroxine and the

[195] I. Perlman, M. E. Morton, and I. L. Chaikoff, *J. Biol. Chem.*, **139**, 433, 449 (1941).

[195a] C. P. Leblond, W. C. Peacock, J. Gross, and R. D. Evans, *Federation Proc.*, **2**, 29 (1943).

[196] C. P. Leblond, J. Gross, W. Peacock, and R. D. Evans, *Am. J. Physiol.*, **140**, 671 (1943/1944).

excretion of iodinated products are increased to about twice the normal rates.

TABLE 149

Administered Radioiodine Taken Up by the Thyroid of the Rat[195]

Time after ^{131}I adminis-tration, hr.	Weight of glands, mg.	Per cent of administered ^{131}I recovered in whole thyroid gland					Per cent of total thyroid ^{131}I found as		
		Total deter-mined (1)	As thy-roxine (2)	As diiodo-tyrosine (3)	As inorganic (4)	Total recovered (2)+(3)+(4)	Thy-roxine	Diiodo-tyrosine	Inorganic
2	14.8	10.8	1.56				14.4		
2	17.1	17.3	3.18				18.4		
2	16.1	12.9	1.73				13.4		
2	20.9	15.3	1.94				12.7		
4	19.3	25.7	4.73	17.40	2.08	24.2	18.4	67.7	8.1
4	15.8	16.2	3.52	11.30	1.41	16.2	21.7	69.8	8.7
4	18.5	13.4	2.47	9.80	1.21	13.5	18.4	73.1	9.0
4	16.5	10.5	1.68	7.39	0.90	9.9	16.0	70.4	8.6
48	11.1	32.4	9.82	17.90	3.18	30.9	30.6	55.2	9.8
48	15.2	28.6	9.63	15.60	3.74	29.0	33.7	54.4	13.1
48	18.8	10.7	3.25	6.70	1.02	10.9	30.4	62.7	9.5
48	19.1	52.2	15.80	31.80	4.17	51.8	30.3	60.9	8.0
96	13.2	24.6	5.61				22.8		
96	10.5	37.3	8.85				23.7		
96	13.5	14.0	3.64				26.0		
96	13.6	15.7	4.04				25.7		

A slower turnover of iodine was observed in nodules than in the glandular tissue of human and dog thyroids.[197]

B. FORMATION OF PROTEIN-BOUND IODINE OF THE PLASMA

Radioactive inorganic iodide injected is converted at an appreciable rate to protein-bound iodine, according to Chaikoff et al.[198] In 13 hours, 50% of the radioiodine content of the plasma of rats maintained on low iodine intake was found to be protein bound, in 25 hours, 90%. In the completely thyroidectomized rat, as was expected, much less, only 5 to 10% of the radioiodine, was found to be bound to proteins as late as 25 hours after the injection. In hyperthyroid animals a striking

[197] C. P. Leblond, I. Davien Puppel, E. Riley, M. Radike, and G. M. Curtis, *J. Biol. Chem.*, **162**, 275 (1946).

[198] I. L. Chaikoff, A. Taurog, and W. O. Reinhardt, *Endocrinology*, **40**, 47 (1947).

increase in the formation of protein-bound iodine above control values was observed as early as 3 hours after administration of radioiodide. At this interval 39 – 73% of the plasma radioiodine of hyperthyroid rats was protein bound, whereas in the controls only 4.1 to 9.2%. Chaikoff *et al.* emphasize that the rate of appearance of protein-bound labeled iodine may prove as useful for testing thyroid activity as the level of protein-bound iodine.

In the investigation of the distribution of radioiodine in the three fractions (inorganic, diiodotyrosine, and thyroxine), nonradioactive fractions were added to the plasma as carriers before subjecting the plasma to butyl alcohol fractionation.[199] The interesting result was obtained that as early as 24 hours after the injection 80% of the radioiodine content of the plasma was found in the thyroxine-like fraction. Since in the thyroid gland the amount of newly formed radioiodotyrosine exceeds that of newly formed radiothyroxine, this finding demonstrates a preferential output of thyroxine into plasma by the gland.

Taurog *et al.*[199a] recently determined the rate of disappearance of protein-bound radioactive iodine present in the plasma by injecting plasma containing labeled protein-bound iodine into the circulation of dogs. Half of the radioactive iodine was found to disappear in 2.8 to 5.2 hours. In dogs weighing 8 to 10 kg., 50 – 100 μg. were calculated to be removed by the tissues in the course of 24 hours and replaced by nonlabeled protein-bound iodine given off by the thyroid. Assuming the thyroid to be almost the only source of the formation of protein-bound iodine of the plasma, and the protein-bound iodine concentration of the plasma to remain constant during the experiment, the amount of protein-bound iodine given off by the thyroid to the circulation in the course of 24 hours works out to be 50–100 μg. The calculation leading to these figures is discussed on page 251.

C. ROLE OF ANTERIOR PITUITARY IN IODINE METABOLISM

1. The Hypophysectomized Animal

At an early stage of the experiment a much smaller amount of a carrier-free dose of radioiodine appears in the thyroid glands of hypophysectomized[199b] than of normal animals. Thus in 4 hours, normal

[199] A. Taurog and I. L. Chaikoff, *J. Biol. Chem.*, **163**, 313 (1946).

[199a] A. Taurog, I. L. Chaikoff, and C. Entenman, *Endocrinology*, **40**, 86 (1947).

[199b] H. Schachner, A. L. Franklin, and I. L. Chaikoff, *Endocrinology*, **34**, 159 (1944).

thyroids contained approximately 60% of injected ^{131}I; at the same time interval the gland of hypophysectomized rats contained less than 5%. Moreover, the character of the curves depicting the uptake of labeled iodine differs in the two types of rats. The gland of the hypo-
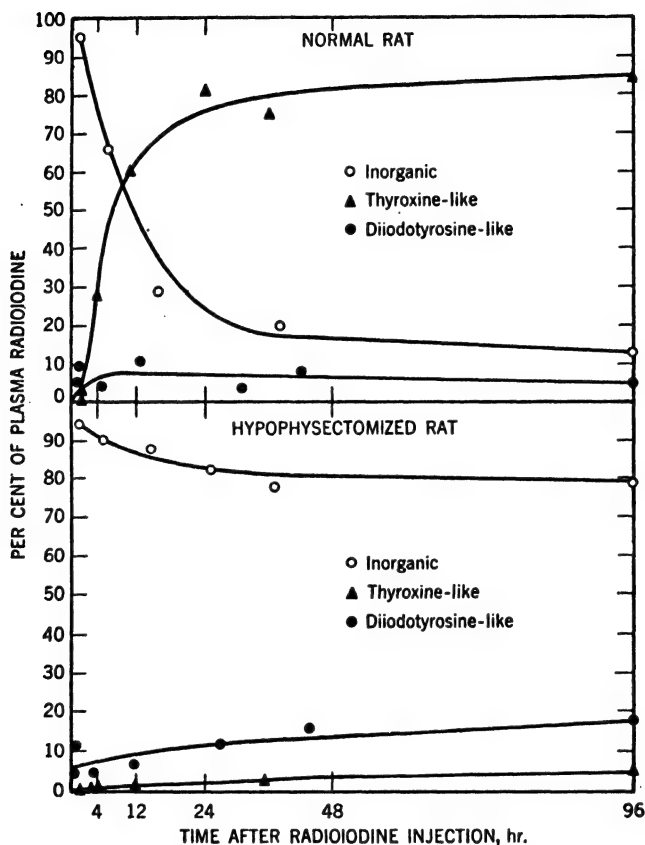
Fig. 69.   Distribution of plasma iodine among different fractions after a single injection of radioactive iodide into normal and hypophysectomized rats (*courtesy* Dr. I. L. Chaikoff).[199c]

physectomized rats should not be regarded as completely inactive, however, for it showed a continuous rise in content of the injected ^{131}I during the entire period of observation and at 96 hours contained about 25% of the administered [131]I.

A striking difference between normal and hypophysectomized rats is also observed in the manner in which injected radioiodine disappeared

[199c] I. L. Chaikoff, *personal communication.*

from the plasma (Figure 69).[199c] Radioiodine leaves the blood more rapidly in the former in keeping with the more rapid uptake of ^{131}I by the thyroids. The rise in the radioiodine content of the plasma of normal rats after 12 hours (not observed in the hypophysectomized rats) reflects the release of labeled hormone from the thyroids.

The conversion of diiodotyrosine to thyroxine appears to be interfered with in the absence of hypophysis. This interpretation of the data is fully supported by the finding that practically none of the plasma radioiodine of the hypophysectomized rat is thyroxine iodine even as late as 96 hours, a time when approximately 80% of the radioiodine contained in plasma of the normal rat is thyroxine iodine. Depression of thyroid function by hypophysectomy results in inhibition of labeled thyroxine formation but not of labeled diiodotyrosine formation from labeled iodide.[199d]

2. The Animal Treated with Thyrotropic Hormone

The thyroid gland of animals injected with thyrotropic hormone have a greater than normal capacity for fixing administered [131]I. This has been shown to be the case whether the radioactive iodine was administered carrier-free or along with carrier. Such increases in uptake have been found in the guinea pig,[200,200a] the rabbit,[201] and the rat.[200,200a]

A study by Morton et al.[200a] has shown that (see Table 150), whereas 5% of an injected tracer dose of ^{131}I was present in the thyroid glands of normal guinea pigs 2 hours after the injection, 12% was found in the hyperplastic glands of pigs treated with thyrotropic hormone. At the 26-hour interval after the injection of radioiodine, 14% of the administered labeled iodine was present in the normal thyroids and 30% in the hyperactive glands. The increased uptake was apparent in all three iodine fractions of the thyroid gland, as shown by Table 150.

In the hyperthyroid animal, as in the normal (cf. Mann et al.[202]), the ^{131}I, when administered as a carrier-free dose, is taken up by the thyroid gland and rapidly converted there to organic iodine. Under the influence

[199d] M. E. Morton, I. Perlman, E. Anderson, and I. L. Chaikoff, *Endocrinology*, 30, 495 (1942).

[200] C. P. Leblond and P. Süe, *Compt. rend. soc. biol.*, 133, 543 (1940).

[200a] M. E. Morton, I. Perlman, and I. L. Chaikoff, *J. Biol. Chem.*, 140, 603 (1941).

[201] S. Hertz, A. Roberts, J. H. Means, and R. D. Evans, *Am. J. Physiol.*, 128, 565 (1940).

[202] W. Mann, C. P. Leblond, and S. L. Warren, *J. Biol. Chem.*, 142, 905 (1942).

of the thyrotropic hormone, the normal distribution of the gland's ^{131}I between thyroxine and diiodotyrosine is changed. In the hyperactive gland a smaller percentage than normal of the gland's ^{131}I was found as diiodotyrosine, whereas a larger percentage than normal was present as thyroxine.

The striking effect of thyrotropic hormone on the metabolic fate of circulating inorganic iodide is shown in Figure 70, which depicts the distribution of the radioactive iodine contained in one of the peripheral tissues (in this case the small intestine) of guinea pigs at various time intervals after the injection of a tracer dose of radioactive iodine. It is assumed here that the movement of radioiodine in and out of the tissues merely reflects the changes in radioiodine concentration of the plasma.[199c]

Fig. 70. Distribution between diiodotyrosine and thyroxine of the radioiodine in the small intestine.[199c]

The application of labeled iodine in the study of thyroid metabolism (*cf.* Taurog *et al.*[202a]) has led to the conclusion that in the thyrotropic hormone-treated animal there is a more rapid removal of circulating radioiodide by the thyroid gland, a more rapid conversion of the radioiodide to thyroxine, and a more rapid release of radiothyroxine.

TABLE 150

Recovery of Trace Dose of Labeled Iodine in Thyroid
and Plasma of Guinea Pigs[200a]

Experimental condition	Time, hr.	Per cent of radioiodine recovered in thyroid gland as		
		Thyroxine	Diiodotyrosine	Inorganic
Normal..........	2	0.44	4.52	0.40
	6	0.58	7.31	0.93
	26	0.97	11.8	0.88
Hyperactive	2	2.38	5.95	3.3
	6	4.23	12.3	4.7
	26	7.17	19.0	3.7

[202a] A. Taurog, I. L. Chaikoff, and L. L. Bennet, *Endocrinology*, **38**, 122 (1946).

Thyrotropic hormones increase while thiourea inhibits the penetration of iodine into the folloicle.[202b]

The rare clinical occurrence of adenocarcinoma of the thyroid concomitant with distant functioning metastases and hyperthyroidism was studied using labeled iodine. The retention of the latter may be taken as a criterion for active thyroid function in the metastatic tissue. In two cases of thyroid adenocarcinoma, hyperthyroidism was shown to be caused by functioning metastases containing labeled thyroxine iodine.[202c]

D. EFFECT OF THIOCYANATE AND THIOURACIL

In thyroids of rats made goitrous by potassium thiocyanate and by thiouracil the uptake of radioactive iodine was found to be reduced.

Two weeks after the animals were placed on a basal diet, a group of rats were given 0.1% thiouracil in the drinking water. Another group was given 0.25% potassium thiocyanate in the drinking water. The remaining 25 animals were maintained on the basal diet alone. On the twenty-eighth day after starting the thiouracil and thiocyanate treatment, all of the animals were injected intraperitoneally with 1 ml. solution containing 2.5 μg. labeled iodide. Four hours later all of the animals were killed. The uptake of radioiodine by thyroid found by Rawson et al.[203] is seen in Table 151.

TABLE 151
Administered Radioiodine in Thyroid Glands of Rats[203]

Group of animals	Mean thyroid weights, mg.	Mean uptake of administered radioactive I, %
Control	15.9	55.7
Thiocyanate treated	29.3	86.5
Thiouracil treated	49.5	10.1

It is evident from the above figures that potassium thiocyanate and thiouracil have a goitrogenic effect. In the hyperplastic thyroids produced by thiouracil the uptake of radioiodine is appreciably lower than in the controls, while the opposite is the case for the thiocyanate-

[202b] C. Chagas, E. De Robertis, and A. Couceiro, *Texas Repts. Biol. Med.*, **3**, 170 (1945).

[202c] L. Leiter, S. M. Seidlin, L. D. Marinelli, and E. J. Baumann, *J. Clin. Endocrinol.*, **6**, 247 (1946).

[203] R. W. Rawson, J. F. Tannheimer, and W. Peacock, *Endocrinology*, **34**, 245 (1944).

TABLE 152

Formation of Thyroxine and Diiodotyrosine by Thyroidectomized Rats[208]

Rat No.	Body weight, g.	Interval after thyroidectomy, month	Oxygen consumption — Per sq. meter per 24 hours	Oxygen consumption — Decrease below normal %	Tissue	Time after injection, hr.	Administered ¹³¹I recovered per g., %	¹³¹I of tissues found as — Thyroxine, %	¹³¹I of tissues found as — Diiodotyrosine, %	¹³¹I of tissues found as — Inorganic, %
131	395	8	97	47	Small intestine	2	0.132	1.5	13.6	87.4
132	385	2	96	48	Small intestine	2	0.143	1.5	9.4	90.0
131					Liver	2	0.0733	1.9	12.6	88.4
132					Liver	2	0.0648	1.7	11.4	85.1
134	290	4	92	50	Small intestine	24	0.110	1.9	20.1	78.4
137	310	8	108	41	Small intestine	24	0.0494	3.9	19.9	69.6
134					Liver	24	0.0692	1.8	20.5	77.5
137					Liver	24	0.0444	4.5	15.2	80.4
134					Muscle	24	0.0408	1.3	8.1	88.0
137					Muscle	24	0.0190	3.3	12.0	83.1
134 } 135 }	285	2	109	41	Plasma	24	0.212	1.0	7.01	91.8
136 } 137]	415	8	101	45	Plasma	24	0.126	0.8	12.2	86.9
140	375	2	95	48	Small intestine	96	0.00583	8.0	13.0	74.7
141	415	2	105	43	Small intestine	96	0.00861	7.2	15.0	77.4
140					Liver	96	0.00563	5.8	21.2	73.7
141					Liver	96	0.00957	8.6	21.3	69.9

treated rats. While the uptake of ^{131}I, by the control thyroids was 56% of the administered tracer dose, that by thyroids of thiocyanate-treated rats, 87%, and that by thiouracil-treated, 10%.[203] Sixteen to twenty-four hours after the last potassium thiocyanate administration, at a time when an increased concentration of the thiocyanate can no longer be demonstrated in plasma, the enlarged whole gland of the thiocyanate-treated rat has a greater than normal capacity for concentrating administered radioactive iodine. This increase is not apparent, however, when the uptake of ^{131}I is calculated on a per-milligram basis.[204] In other experiments the daily feeding of 3.4 to 4.2 mg. of thiouracil for seven days was found to depress the uptake of injected ^{131}I by the thyroid gland of the rat. The recovery of the gland as judged by its capacity to remove injected radioiodines, was complete in two weeks.[205]

Thiouracil was also found to inhibit uptake of radioactive iodine by thyroid of normal chick and by hyperplastic chicks.[206] Administration of thiourea, adding to the diet 1% of thiourea for one month, was found to obstruct the accumulation of radioiodine in any form in the thyroid of the rat.[207]

E. FORMATION OF DIIODOTYROSINE AND THYROXINE IN THYROIDECTOMIZED ANIMALS

Does formation of diiodotyrosine and thyroxine take place in thyroid-ectomized animals? This point has been investigated by Morton et al.[208] making use of rats thyroidectomized at the age of 4 to 6 months and maintained on a stock diet for 2 to 8 months. Their basal metabolic rate was $40-50\%$ below normal. Tracer doses of ^{131}I were administered and the various organs analyzed for their thyroxine, diiodotyrosine, and inorganic iodine content. All organs investigated were found to contain these compounds, as is seen in Table 152, the small intestine containing a much larger amount of radiothyroxine after 24 than after 2 hours. As early as 96 hours after its injection, 30% of the radioiodine contained in

[204] I. Wolff, I. L. Chaikoff, A. Taurog, and L. Rubin, *Endocrinology*, **39**, 140 (1946).

[205] A. L. Franklin, S. R. Lerner, and I. L. Chaikoff, *Endocrinology*, **34**, 265 (1944).

[206] R. A. Larson, F. R. Keating, Jr., W. Peacock, and R. W. Rawson, *Endocrinology*, **36**, 149, 160 (1945).

[207] A. S. Keston, E. D. Goldsmith, A. S. Gordon, and H. A. Charipper, *J. Biol. Chem.*, **152**, 241 (1944).

[208] M. E. Morton, I. L. Chaikoff, W. O. Reinhardt, and E. Anderson, *J. Biol. Chem.*, **147**, 757 (1943).

the liver and in the small intestine was organically bound, 20% as diiodotyrosine and 8% as thyroxine. The ratio between inorganic and organic iodine was, however, much larger in the different organs than in the case of nonthyroidectomized rats.

Thyroxine and diiodotyrosine extracted from the organs were recrystallized five times. In each recrystallization of thyroxine, 10 mg. of nonradioactive diiodotyrosine were added in order to wash out any contaminating radiodiiodotyrosine by dilution. Such a washing-out process is much used in the purification of labeled compounds. In the case of diiodotyrosine recrystallization, 10 mg. of nonradioactive thyroxine were added each time for the same purpose.

The specific activities of both thyroxine and diiodotyrosine did not change significantly after the second recrystallization. This would be expected to occur only in a case in which the substance giving the radioactivity was identical with the material that underwent repeated recrystallization.

The completeness of thyroidectomy was checked with great care by histological examination of serial sections of the whole neck and chest regions of the animals, and in some cases by a radioautographic method in which the tissues are rolled out and exposed to an x-ray film. Under such circumstances a high concentration of radioactive iodine such as occurs in residual fragments of thyroid tissue is automatically revealed. Finally, the results of the experiments were not in any way influenced by previous hypophysectomy as would have been expected if they had been due to undetected thyroid tissue. The thyroidectomy was carried out very carefully and controlled by radioautographs. Chaikoff and co-workers also found that the administered ^{131}I can be recovered almost quantitatively from the tissue.

At first sight it may be astonishing to find the formation of thyroxine in a thyroidectomized organism, but this is in no way surprising in view of the discoveries of Mutzenbecher[209] and others. By iodination of casein, they obtained preparations that were physiologically active and from which thyroxine could be isolated. Harington and others obtained similar results.[210]

F. FORMATION OF DIIODOTYROSINE AND THYROXINE IN TISSUE SLICES

That the thyroid gland has an extraordinary capacity for accumulating iodine is demonstrated by experiments with tissue slices.[211,212] At

[209] P. v. Mutzenbecher, *Z. physiol. Chem.*, **261**, 253 (1939).

[210] C. R. Harington, *Proc. Roy. Soc. London*, **B132**, 223 (1944).

[211] M. E. Morton and I. L. Chaikoff, *J. Biol. Chem.*, **144**, 565 (1942).

[212] M. E. Morton and I. L. Chaikoff, *J. Biol. Chem.*, **147**, 1 (1943).

the end of 2 hours' incubation in bicarbonate–Ringer solution containing tracer-doses of radioiodide, 81% of the ^{131}I was recovered in 300 mg. surviving slices of thyroid gland of sheep, but only 2% in slices of liver, as is seen in Table 153. In experiments in which the Ringer solution

TABLE 153

Accumulation of ^{131}I in One Hour in Surviving Liver and Thyroid Slices of Sheep[211]

| Tissue | Per cent of Ringer ^{131}I recovered in slices as | | |
	Organic	Inorganic	Total
Liver..............	0.1	2.6	2.7
Liver..............	0.0	2.1	2.1
Liver..............	0.1	2.2	2.3
Thyroid...........	57.5	21.5	79.0
Thyroid...........	56.9	22.1	80.0
Thyroid...........	63.4	21.9	85.3

contained 0.1 g. labeled iodine, after a lapse of three hours, 12% of the ^{131}I was found to be present in the thyroxine and 70% in the diiodo tyrosine fractions. Homogenization or desiccation of the tissue was found by Chaikoff and his associates to prevent the conversion of radioiodine to diiodotyrosine and thyroxine.[212] The process of conversion is retarded by poisons known to inhibit aerobic oxidation involving the cytochrome oxidase system (potassium cyanide, hydrogen sulfide, carbon monoxide, and sodium azide) (see Table 153A). Anaerobiosis causes a strong inhibition when the amount of oxygen is reduced below 0.6%.[213] The inhibition by carbon monoxide is more pronounced in the dark than in the light (see Table 153A).

In order to study the mechanism of action of various known goitrogenic compounds, Chaikoff and colleagues investigated the effects of the compounds on *in vitro* conversion of radioactive inorganic iodide to thyroxine and diiodotyrosine by thyroid slices as well. Thiouracil and allylthiourea were found to be effective inhibitors even at concentrations as low as 10^{-4} M.[214] Similar effects are produced by sulfanilamide, sulfapyridine, sulfaguanidine, and sulfathiazole. These substances, at a concentration of 10^{-3} M, inhibit the formation of radioiodotyrosine and

[213] H. Schachner, A. L. Franklin, and I. L. Chaikoff, *J. Biol. Chem.*, **151**, 191 (1943).
[214] A. L. Franklin, I. L. Chaikoff, and S. R. Ler er, *J. Biol. Chem.*, **153**, 151 (1944).

radiothyroxine.[215]   Numerous compounds structurally related to the sulfonamides and aminobenzoic acid were tested for their effects on the *in vitro* conversion of radioactive iodide, to thyroxine and diiodotyrosine by surviving tissue slices.   A free aromatic amino or hydroxyl group was

TABLE 153A

Effect of Various Inhibitors on Synthesis[213] of Thyroxine and Diiodotyrosine by Surviving Thyroid Tissue Slices as Measured with ^{131}I

Inhibitor	Concentration in Ringer's solution or in atmosphere above solution	Per cent inhibition	
		Thyroxine formation	Diiodotyrosine formation
Respiratory inhibitors			
Azide..................	0.005 M	90	89
Cyanide...............	0.01 M	94	83
Sulfide...............	0.003 M	89	85
Anaerobiosis...........	95% N_2	67	54
	5% CO_2		
Carbon monoxide........	90% CO	71 (in dark)	81 (in dark)
	5% CO_2	46 (in light)	39 (in light)
	5% O_2		
Goitrogenic substances			
Thiouracil.............	0.001 M	95	90
Sulfanilamide..........	0.001 M	47	58
p-Aminobenzoic acid.....	0.001 M	73	79

found to favor inhibitory activity, while the presence of such groups as the sulfonamide, the sulfonic acid, or the carboxyl was found to be unrelated to activity.   The results obtained indicate a correlation between ease of oxidizability and inhibitory activity among the compounds tested.[216]   The accumulation of inorganic iodine by surviving thyroid tissue, as measured with radioactive iodine, is inhibited in the presence of $10^{-3} M$ KSCN.   Under these conditions, the drug also depresses the synthesis of diiodotyrosine and thyroxine.[204]

When, instead of adding tracer doses of labeled iodine to Ringer solution, 10 μg. or more was added, an inhibition of the formation of labeled thyroxine and diiodotyrosine in slices of sheep thyroid at the expense of inorganic iodide of the medium was observed, as seen in Table 154.   The inhibiting effect of iodine on conversion of inorganic

[215] A. L. Franklin and I. L. Chaikoff, *J. Biol. Chem.*, **152**, 295 (1944).
[216] A. Taurog, I. L. Chaikoff, and A. L. Franklin, *J. Biol. Chem.*, **161**, 537 (1945).

iodide to thyroxine and diiodotyrosine is possibly due to iodination of the enzymes concerned.

Cyanide and sulfide, in addition to completely inhibiting formation of thyroxine and diiodotyrosine, markedly depress accumulation of [131]I

TABLE 154

Inhibitory Effect of Added Inorganic Iodine on Formation of Labeled Thyroxine and Diiodotyrosine[217]

Thyroid tissue in flask, mg.	Inorganic iodide [127]I added to flask, γ	Per cent of Ringer ^{131}I recovered as			Ringer [127]I converted to	
		Thyroxine	Diiodo-tyrosine	Inorganic	Thyroxine, γ	Diiodo-tyrosine, γ
300	0.3	10.6	45.6	43.8	0.03	0.14
300	0.3	8.7	42.9	48.4	0.03	0.13
305	20.3	2.2	6.1	91.7	0.45	1.2
305	20.3	2.1	6.9	91.0	0.43	1.4
305	50.3	0.50	2.4	97.1	0.25	1.2
300	50.3	0.40	2.4	97.2	0.20	1.2

by thyroid slices. Despite the almost complete inhibition of thyroxine and diiodotyrosine formation by 10^{-3} M azide, as much as 60% of the Ringer solution iodide entered the tissue slices. Sulfanilamide, which strongly inhibits the *in vitro* conversion of Ringer iodide to thyroxine and diiodotyrosine, has at a concentration of 10^{-3} M little effect on the iodine-concentrating capacity of thyroid tissue. From these and similar facts Chaikoff and co-workers[218] conclude that in the thyroid tissue there exists a mechanism for concentrating iodine that does not depend upon the conversion of inorganic iodide to thyroxine and diiodotyrosine.

Biological iodination was found to take place in 5 ml. unpasteurized milk to which, beside 0.5 μg. radioiodide, a phosphate buffer and 0.45 mg. xanthine were added. In the course of 45 minutes at 38°C. about half the iodide added was no longer present in the inorganic iodine fraction. The presence of thiourea inhibits the effect of xanthine.[219]

After it had been found, by making use of radioiodine as a tracer, that

[217] M. E. Morton, I. L. Chaikoff, and S. Rosenfeld, *J. Biol. Chem.*, **154**, 381 (1944).

[218] H. Schachner, A. L. Franklin, and I. L. Chaikoff, *Endocrinology*, **34**, 159 (1944).

[219] A. S. Keston, *J. Biol. Chem.*, **153**, 335 (1944).

diiodotyrosine and thyroxine were formed in tissue slices placed in bicarbonate – Ringer, the formation of these compounds was also demonstrated by Harington[210] with the usual analytical methods. He found that both diiodotyrosine and thyroxine were formed in the presence of iodine; but, in the absence of iodine in a medium containing diiodotyrosine and thyroid slices, no increase of thyroxine could be observed. This suggests that the essential biochemical reaction may be the liberation of iodine from iodide by an oxidizing enzyme system. The enzymic reaction is concerned with the formation of diiodotyrosine.

G. FATE OF INJECTED LABELED THYROXINE

Joliot and associates[220-222] applied the methods of Harington and Barger in the preparation of labeled thyroxine and compared the uptake and excretion of labeled thyroxine injected intravenously to rabbits with the behavior of injected labeled iodide. Up to fifty times as much

TABLE 155

Activity Figures for the Uptake and Excretion of Radioiodine by Rabbits after Administration of Labeled Sodium Iodide and Labeled Thyroxine[221]

Organ	11.7 mg. labeled I injected as NaI			7.8 mg. labeled I injected as thyroxine		
	Iodide	Diiodotyrosine	Thyroxine	Iodide	Diiodotyrosine	Thyroxine
Urine........	13.4	10	0.1	5.6	8.3	8.25
Thyroid.....	312.5	59	2.9	20.9	8.5	1.7
Fetus.......	7.2	7	1.1	0.23	0.35	0.45
Blood.......	2.3	2.5	0.2	0.42	0.5	1.5

labeled thyroxine iodine as labeled iodide is found to be excreted through the bile. The thyroid gland takes up appreciably more radioiodine after administration of radioiodide than after administration of radiothyroxine, as may be seen in Table 155. The uptake of labeled thyroxine per gram of hypophysis tissue was found to be larger than the uptake of labeled iodide.

[220] C. P. Leblond, P. Süe, and A. Chamorro, *Compt. rend. soc. biol.*, **133**, 540 (1940).
[221] F. Joliot, R. Courrier, A. Horeau, and P. Süe, *Compt. rend.*, **218**, 769 (1944).
[222] F. Joliot, *Proc. Roy. Soc. London*, **A184**, 1 (1945).

XIII. Turnover of Carbon Compounds

A. GENERAL REMARKS

Among the radioactive isotopes of carbon, before ^{14}C became easily available, ^{11}C was much used as a tracer. The short-lived isotope ^{11}C has the advantage that very active samples free of stable C isotopes can be obtained, mostly because of the short half-life. Large doses can be administered without producing noxious effects. Due to the hardness of the β-rays emitted, the activity of this isotope can be measured easily. Results can be obtained almost at once by using this tracer.

The short half-life of about 21 minutes imposes severe restrictions on the application of ^{11}C, however. For the same reason ^{11}C can be employed only by laboratories close to the means of production. ^{14}C, having a half-life of about 5100 years and being available in relatively large amounts at present, will be much preferred to ^{11}C in experiments involving lengthy chemical procedures.

Radiocarbon has been administered in the form of carbon dioxide, bicarbonate, lactate, or other organic compounds. In view of the short life of ^{11}C ($T = 21$ minutes), special methods were devised for the synthesis of organic compounds containing this isotope. Methods of synthesis both *in vivo* and *in vitro* were used. In experiments with methane bacteria, 40 minutes of exposure to about 2 cc. labeled carbon dioxide resulted in the transformation of a large fraction of the labeled carbon dioxide into active methane. In the presence of glycerol, *Propioni-bacterium pentosaceum* reduced 80% of a similar quantity of carbon dioxide to propionic and succinic acids in 30 minutes. *Clostridium acidiurici*, in fermenting uric acid, reduced appreciable quantities of carbon dioxide in 15 minutes to active acetic acid. Using hypoxanthine as substrate, 80 minutes sufficed to convert nearly all the carbon dioxide into acetic acid. It was found that both methyl and carboxyl groups were labeled.[223] Photosynthetic organisms can furthermore be used for the production of carbohydrates from carbon dioxide. If barley is employed, it is possible to transform about 20% of a given quantity of carbon dioxide into sugar, as was shown by Carson and Ruben.[223] The protozoan *Tetrahymena geleii* ferments glucose anaerobically to lactic, acetic, and succinic acids. When this organism is cultured in the presence of a few milliliters of labeled carbon dioxide,

[223] S. F. Carson and S. Ruben, *Proc. Natl. Acad. Sci. U. S.*, **26**, 422 (1940).

thirty minutes of fermentation suffice to incorporate up to 50% of the labeled carbon into the carboxyl group of succinic acid.[223a]

As examples of synthesis *in vitro* we shall consider the formation of labeled acetic, propionic, and butyric acids. The Grignard reaction was used in these syntheses according to the following equations:

$$RI + Mg \rightarrow RMgI$$
$$RMgI + {}^{11}CO_2 \rightarrow R^{11}COOMgI$$
$$R^{11}COOMgI + HOH \rightarrow R^{11}COOH + MgI(OH)$$
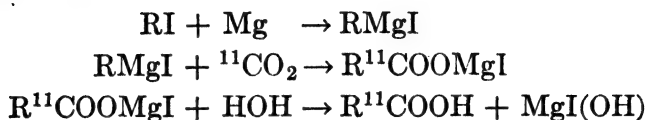
where R stands for the radical $CH_3—$, $C_2H_5—$, or $C_3H_7—$. Good yields of fatty acids based on the $^{11}CO_2$ used were obtained. In the case of acetate, actual measurements of the over-all yield demonstrated that 50% of the $^{11}CO_2$ was converted into acetic acid during the synthesis.

Radioactive acetylene is obtained by reducing labeled barium carbonate to barium carbide and subsequent hydrolysis. By this method a yield of 50 to 70% radioactive acetylene could be obtained in about 10 minutes.[224]

The synthesis of radioactive lactic acid of type I was carried out by way of the following stages ($CH_3CHOH^{11}COOH$ is designated type I lactate, and $^{11}CH_3CHOHCOOH$ is called type II by Vennesland and associates.[225]):

$$^{11}CO_2 + 4\,K + NH_3 \rightarrow K^{11}CN + 2\,KOH + KH$$
$$H^{11}CN + CH_3CHO \rightarrow CH_3CHOH^{11}CN$$
$$CH_3CHOH^{11}CN + H_2O \rightarrow CH_3CHOH^{11}COOH$$
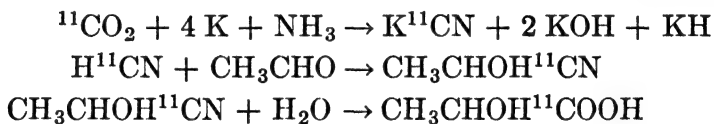
The synthesis required 45 to 60 minutes. The subsequent purification and preparation of the lactic acid for biological experiments took another 45 to 60 minutes.

The synthesis of lactic acid of type II results in the formation of a product which is probably a nearly equivalent mixture of

[223a] C. B. Van Niel, J. O. Thomas, S. Ruben, and M. D. Kamen, *Proc. Natl. Acad. Sci. U. S.*, **28**, 157 (1942).

[224] B. Vennesland, A. K. Solomon, J. M. Buchanan, R. D. Cramer, and A. B. Hastings, *J. Biol. Chem.*, **142**, 371 (1942). R. D. Cramer and G. B. Kistiakowsky, *ibid.*, **137**, 549 (1941).

[225] B. Vennesland, A. K. Solomon, J. M. Buchanan, and A. B. Hastings, *J. Biol. Chem.*, **142**, 379 (1942).

$CH_3{}^{11}CHOHCOOH$ and $^{11}CH_3CHOHCOOH$. It consists of the following stages:

$$Na_2{}^{11}CO_3 + BaCl_2 \rightarrow Ba^{11}CO_3 + 2\ NaCl$$
$$2\ Ba^{11}CO_3 + 6\ Mg \rightarrow Ba^{11}C_2 + 6\ MgO$$
$$Ba^{11}C_2 + H_2O \rightarrow H^{11}C \equiv {}^{11}CH$$
$$H^{11}C \equiv {}^{11}CH + H_2O \rightarrow {}^{11}CH_3{}^{11}CHO$$
$$H^{11}C \equiv {}^{11}CH + H_2O \rightarrow {}^{11}CH_3{}^{11}CHO$$
$$CH_3{}^{11}CHO + KCN \rightarrow CH_3{}^{11}CHOHCN$$
$${}^{11}CH_3CHO + KCN \rightarrow {}^{11}CH_3CHOHCN$$
$$CH_3{}^{11}CHOHCN + H_2O \rightarrow CH_3{}^{11}CHOHCOOH$$
$${}^{11}CH_3CHOHCN + H_2O \rightarrow {}^{11}CH_3CHOHCOOH$$
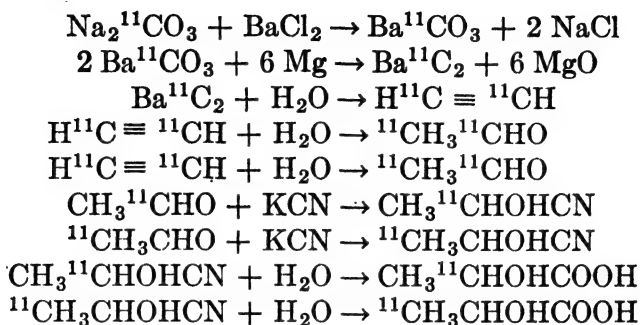
The synthesis of the acid required about 105 minutes; purification and preparation for biological experiments needed a further 45 minutes. At the end of the synthesis some 40 to 50% of the original radioactive carbon was present in lactic acid, allowance being made for the disintegration which occurred meanwhile.

The radioactive carbon dioxide was obtained from the cyclotron by pumping the gas $^{11}CO_2$ from the target chamber through 3 ml. of a

Fig. 71. (I) Vessel used to collect radioactive carbon dioxide (A, pressure bulb; B, reservoir containing mercury; C, outlet to Van Slyke apparatus or reaction vessel). (II) Reaction vessel used for synthesis of radioactive fatty acids.[226]
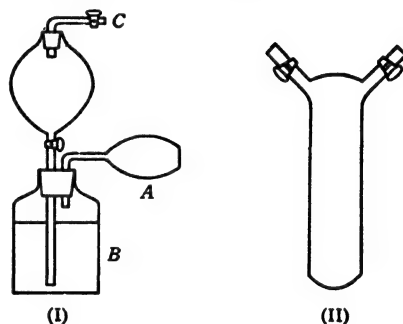
solution of 2 M sodium hydroxide and 2/3 M sodium carbonate. The collecting tube was chilled in liquid air to secure efficient collection of the $^{11}CO_2$. Upon being warmed, the alkaline solution was transferred to the reaction chamber, of a Van Slyke manometric gas apparatus and the solution was acidified with 4 ml. of 5 N lactic acid. The carbon dioxide evolved was collected over mercury in a collecting vessel (Figure 71, I) which was designed so that the gas could be returned to a reaction vessel (Figure 71, II) containing the Grignard reagent.[226] Irradiation

[226] J. M. Buchanan, A. B. Hastings, and F. B. Nesbett, *J. Biol. Chem.*, **150**, 413 (1943).

of ammonium nitrate by neutrons in the cyclotron results in the formation of various radioactive carbon (^{14}C) compounds such as CO, CO_2, CH_4, CH_3OH, and $HCOOH$.[226a]

B. FORMATION OF GLYCOGEN

1. Administration of Labeled Lactate

The carbon of the glycogen formed following the administration of lactate to an animal may conceivably come from (a) the three carbon atoms of the administered lactate, (b) a two-carbon residue left after

TABLE 156

Radioactivity of Liver Glycogen 2.5 Hours after Feeding
^{11}C Lactate to the Rat[227]

Expt. No.	Rat weight, g.	Lactate		Liver		Glycogen					Ratio, (2) : (1)
		Fed, mg.	Absorbed, mg.	Weight, g.	Glycogen, %	Formed,[a] mg.	Per cent of lactate fed (1)	Radioactivity			
								Per cent of amount fed (2)	Error, %		
1	115	44	——	——	——	——	—	1.7	3		—
2	115	30	——	——	——	——	—	1.0	43		—
3[b]	136	130	——	——	——	——	—	0.7	19		—
4	108	150	——	4.30	1.72	69	46	2.1	5		0.05
5	100	50	50	3.60	0.77	23	46	2.4	18		0.05
6	111	109	104	4.63	1.88	81	74	1.8	21		0.02
7	122	214	81	4.44	1.09	43	20	1.3	13		0.06
8	140	196	117	5.24	0.46	18	9	3.1	3		0.34
9	140	250	171	5.33	0.67	29	12	1.4	5		0.12
10	121	162	126	4.65	0.72	28	17	0.7	3		0.04
Average							32	1.6			0.10

[a] Corrected for amount found in livers of fasted controls (0.12%).
[b] Two-hour experiment.

decarboxylation of the lactate molecule, (c) other glycogen precursors already present or formed in the organism, or (d) carbon dioxide produced in metabolic activity. Conant and colleagues[227] used radioactive

[226a] P. E. Yankwich, G. K. Rollefson, and T. H. Norris, J. Chem. Phys., 14, 131 (1946).
[227] J. B. Conant, R. D. Cramer, A. B. Hastings, F. W. Klemperer, A. K. Solomon, and B. Vennesland, J. Biol. Chem., 137, 557 (1941).

carbon (^{11}C) in an attempt to provide information regarding the source or sources of glycogen carbon. If the newly formed glycogen contained radioactivity in the same proportion as in the administered lactate, it might be concluded that the glycogen was actually derived from the lactate administered (source *a*). If the radioactivity of the newly formed glycogen were significantly less, the alternative sources *b* and *c* would also have to be considered. The data obtained (see Table 156) indicate, in fact, that the three carbon atoms of the lactate molecule were not the sole source of the carbon of the new liver glycogen.

TABLE 157

Excretion of ^{11}C in Urine and Expired Carbon Dioxide by the Rat[227]

Experiment No.	Radioactivity in expired CO₂							Radioactivity in urine, per cent of amount fed
	Per cent of amount fed					Total per cent of amount fed	Total per cent of amount absorbed	
	0.5 hr.	1.0 hr.	1.5 hr.	2.0 hr.	2.5 hr.			
1	2.3	7.7	9.0	11.2	9.3	39.5	——	——
2	1.2	4.6	6.8	5.2	4.2	22.0	——	3.5
3ᵃ	1.0	4.9	8.1	4.2	—	18.2	——	1.4
4	2.0	7.5	11.6	10.4	11.5	43.0	——	1.6
5	1.3	3.0	4.1	4.5	3.0	15.9	15.9	——
6	0.6	1.2	4.1	3.9	—	9.8	10.3	——
7	0.7	1.7	2.3	4.5	3.2	12.4	32.8	——
8	0.6	1.9	3.0	3.5	4.5	13.5	22.6	——
9	0.7	1.7	4.2	2.6	1.2	10.4	15.2	——
10	0.4	1.2	3.3	3.6	2.5	11.0	14.1	——
Average	*1.1*	*3.5*	*5.6*	*5.4*	*4.9*	*20*		

ᵃ Two-hour experiment.

The promptness with which radioactivity appeared in the exhaled carbon dioxide (Table 157) indicates that the lactate was readily absorbed and, to some extent, metabolized within the first half-hour after its administration. The rate of excretion of radioactive carbon dioxide increased during the second and third half-hour periods to an average of about 5% of the radioactivity administered per half-hour period. Radioactivity was still appearing in the expired carbon dioxide at approximately the same rate when the animals were killed at the end of 2.5 hours. The amount of expired carbon dioxide derived from the

lactate administered constituted less than one-thirteenth of the total carbon dioxide expired.

The amount of radioactivity found in the glycogen was only 1.6% of the administered radioactivity, although the amount of liver glycogen formed, in terms of the lactate fed, would appear to account for 32% of the lactate. This fact eliminates the unbroken three-carbon chain of the lactate molecule as the principal source of glycogen. The results may be interpreted as an effect of "dilution" of the labeled three-carbon chain in the animal body. They do not necessarily indicate that the carboxyl group is detached from the rest of the carbon chain in the course of glycogen synthesis.

Vennesland et al.[225] compared these results with those of analogous experiments employing lactate containing ^{11}C in the α- or β-position (type II lactate) to determine whether the latter step actually occurred. They found that the liver glycogen formed averaged 21% of the lactate fed and contained 3.2% of the radioactivity, i.e., a definitely higher amount than that previously found with type I. The labeled carbon dioxide expired in 2.5 hours accounted for only 10% of the administered radioactivity. This result substantiated the view that the fate of the carboxyl carbon atom of lactic acid was quite different from that of the α- and β-carbon.

In other experiments,[228] nonradioactive lactate was fed to fasting rats and one-fifth of the labeled bicarbonate solution was injected intraperitoneally immediately after the lactate had been administered. The four additional portions were then injected at successive half-hour intervals. The expired carbon dioxide was also collected at half-hour intervals; finally, after 2.5 hours, the glycogen was isolated. The ^{11}C content of the fractions collected is given in Table 158. About 39% of the ^{11}C was not accounted for.

According to the view advanced by the Harvard group,[225] the original labeled α- or β-carbon atom of the lactate (type II) will be incorporated with glycogen but it must be taken into consideration that the specific activity of the labeled substances introduced is diminished by dilution or interchange reactions in the body. This diminution in the specific activity may occur at any of the steps involved. When the carboxyl carbon is labeled, as in the type I lactate experiments, the lactate will be diluted by the same amount as in the type II lactate

[228] A. K. Solomon, B. Vennesland, F. W. Klemperer, F. W. Buchanan, and A. B. Hastings, J. Biol. Chem., 140, 171 (1941).

experiment; but after distribution of the ^{11}C in both carboxyl groups of dicarboxylic acids, half of the labeled atoms will be lost in the subsequent decarboxylation. Consequently, the glycogen formed after feeding type I lactate should contain about half as much ^{11}C as that formed after feeding type II lactate. This is the order of magnitude of the ratio actually observed.

TABLE 158

Recovery of ^{11}C Administered as Bicarbonate to Rats[228]

Fraction	Per cent of ^{11}C administered
Expired $^{11}CO_2$	52.2
Unabsorbed $NaH^{11}CO_3$	0.5
Urine $NaH^{11}CO_3$	1.3
Bone $^{11}CO_2$	1.8
Liver glycogen ^{11}C	0.7
Body fluids ^{11}C	5.0
Total ^{11}C accounted for	*61.5*

Solomon and co-workers[228,229] emphasize that the fixation reaction 1 plays an important part in glycogen synthesis by providing a means for circumventing the only irreversible reaction in glycolysis, *i.e.*, the conversion of phosphopyruvic acid into pyruvic acid. The mechanism of this circumvention is represented empirically by the following steps (*cf.* Wood[230]).

$$CH_3COCOOH + {}^{11}CO_2 \rightleftharpoons {}^{11}COOHCH_2COCOOH \qquad (1)$$

$${}^{11}COOHCH_2COCOOH \rightleftharpoons {}^{11}COOHCH:CHCOOH \qquad (2)$$

$${}^{11}COOHCH:CHCOOH \rightarrow CH_2:C(OPO_3H_2){}^{11}COOH + CO_2 \qquad (3)$$

$$\text{or}$$

$$CH_2:C(OPO_3H_2)COOH + {}^{11}CO_2$$
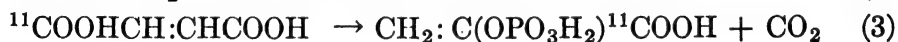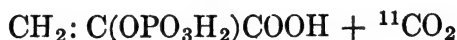
The two types of phosphopyruvic acid with respect to labeled carbon partaking in reaction 3 are a consequence of the symmetrical nature of the dicarboxylic acid formed in reaction 2. These considerations lead to the conclusion that the resulting glycogen should contain one atom of ^{11}C. From the specific activity of the expired carbon dioxide carbon and the specific activity of the newly formed glycogen carbon it was

[229] J. M. Buchanan, "Glycogen Formation," *Thesis*, Harvard Univ., 1943, p. 208.
[230] H. G. Wood, *Physiol. Revs.*, **26**, 198 (1946).

estimated and found to average 11%. An alternative interpretation of the above results is discussed by Wood.[230] Recently, it was reported[232] that direct phosphorylation of pyruvate to form phosphopyruvate can occur in extracts of rat muscle in the presence of potassium ions.

2. Administration of Labeled Bicarbonate

In these experiments[224] fasted rats were fed 300 to 600 mg. glucose in 2 ml. water and injected with $NaH^{11}CO_3$ solution at half-hour intervals. The liver glycogen formed in the course of 2.5 hours incorporated an amount of ^{11}C corresponding to 13% of the carbon atoms of the glycogen, as seen in Table 159.

TABLE 159

Proportion of Radioactivity Expired and of Glycogen Carbon
Derived from Labeled Carbon in 2.5 Hours[224]

Experiment No.	Radioactivity in expired CO_2, per cent of total amount injected	Labeled carbon incorporated into glycogen, mM	Glycogen carbon derived from labeled carbon, %
1	52.5	0.339	8.1
2	54.4	0.495	17.3
3	73.7	0.204	6.6
4	58.8	0.146	20.4
5	74.2	0.108	13.2
Average.........	62.7	0.258	13.1

When radioactive bicarbonate was injected into a well-fed rat with large stores of liver glycogen, no radioactivity was found in the liver glycogen isolated after 2.5 hours. Thus, the incorporation of ^{11}C into liver glycogen occurs to an appreciable extent only when the animal is actually depositing glycogen. A significant amount of ^{11}C was also found in the muscle glycogen of the rats, the radioactivity of this glycogen amounting to about one-third of that in liver glycogen. This was not the case after feeding lactate or pyruvate. The fact that the radioactive carbon of injected $NaH^{11}CO_3$ is incorporated into glycogen to the same extent after glucose feeding as after lactate and pyruvate feeding indicates, according to Buchanan and Hastings,[231] that the

[231] J. M. Buchanan and A. B. Hastings, *Physiol. Revs.*, **26**, 120 (1946).
[232] H. A. Lardy and I. A. Ziegler, *J. Biol. Chem.*, **159**, 343 (1945).

glycogen deposited during the assay period has originated in part, at least, from compounds which have undergone a carbon dioxide incorporation reaction. Since the glucose absorbed must have represented a significant portion of the final metabolic pool, it must be assumed that the ingested glucose very rapidly comes into equilibrium with the dicarboxylic acid pool before its conversion into glycogen.

A study has also been made of glycogen synthesis in muscle tissue after feeding lactate, pyruvate, or glucose and injecting $NaH^{11}CO_3$. After the administration of glucose and $NaH^{11}CO_3$, the muscle glycogen contained significant amounts of ^{11}C.[232] This was not the case after feeding lactate or pyruvate.[228] The failure of ^{11}C to appear in muscle glycogen after the administration of lactate may be due either to a lack of glycogen synthesis in this tissue during the experiment or to a difference in the mechanism of glycogen synthesis in muscle and liver. In interpreting this finding, the possibility of low permeability of the muscle to ^{11}C-containing glycogen precursors is also to be considered.

According to Buchanan and Hastings,[231] it is possible that two paths of glycogen synthesis from lactate occur, the one supplementary to the other. In certain tissues such as muscle, where glycogen synthesis from lactate does not take place at a rapid rate and where carbon dioxide assimilation reactions are not particularly marked, the predominant pathway of glycogen formation may be the direct conversion of pyruvate into phosphopyruvate. In liver tissue, however, where glycogen synthesis from lactate is rapid, glycogen formation involving the synthesis of dicarboxylic acids and the incorporation of carbon dioxide may be the primary pathway.

3. Administration of Labeled Fatty Acids

In experiments with labeled fatty acids, about 4 ml. of a 1.5 mM solution of the sodium salt of a fatty acid containing ^{11}C, to which 400 mg. glucose had been added, was fed to a rat previously fasted for 24 hours.[226] The animal was placed in the metabolism cage and the respiratory gases were collected every half-hour for a period of 2 hours. At the end of this period, the animal was sacrificed and the liver glycogen isolated. The rapid metabolism of the fatty acids is demonstrated by the fact that in these experiments about 50% of the radioactive fatty acid absorbed was excreted in the respiratory gases as carbon dioxide over a 2-hour period, as seen in Table 160. The total radioactivity, on the average, amounted to 4.3% of that of the radiocarbon

absorbed after administering "carboxyl-radioactive" propionate, but was only 1.4% after administration of "carboxyl-radioactive" acetate.

TABLE 160

Excretion of ^{11}C in Expired Carbon Dioxide after Feeding "Carboxyl-Radioactive" Propionate and 400 Mg. Glucose[226]

Expt. No.	CO2 expired	Radioactivity in expired CO2, per cent of amount fed					Total ^{11}C, per cent of amount absorbed
		0.0 to 0.5 hr.	0.5 to 1.0 hr.	1.0 to 1.5 hr.	1.5 to 2.0 hr.	Total	
1	14.2	4.5	8.2	11.7	6.7	31.1	42.0
2	13.9	2.7	4.4	5.5	6.7	19.3	42.9
3[a]	15.5	4.3	7.8	9.1	9.4	30.6	56.6
4	16.1	3.0	6.4	7.7	7.1	24.2	66.6
5	11.5	3.4	6.2	4.2	5.2	19.0	44.9
6	12.6	4.6	6.7	6.8	8.1	26.2	76.9
Av.	13.7	3.6	6.4	7.2	6.8	24.0	54.8

[a] Omitted from average.

The experiments described were conducted on the hypothesis that, if any of the administered fatty acids were oxidized via pathways
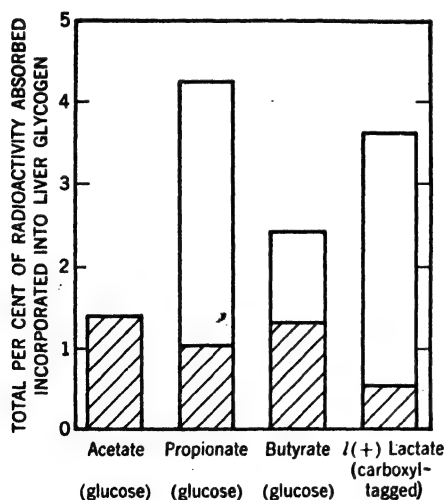
Fig. 72. Comparison of radioactivity present in newly formed liver glycogen following administration of glucose plus carboxyl-labeled acetate, propionate, and butyrate.[226] Results from labeled lactate experiments are included at the right. The heights of the columns indicate total radioactivity present. The shaded areas indicate radioactivity of glycogen due to incorporation of [11]CO2 derived from the metabolism of the acids.

involving carbohydrate intermediates or compounds with which the carbohydrates were in dynamic equilibrium, the isotopic carbon of the

fatty acid fed should be found in newly formed glycogen carbon. All of the radioactivity of the glycogen carbon after acetate feeding could be accounted for by the incorporation of $^{11}CO_2$ derived from the metabolism of the labeled acetate (see Figure 72). The glycogen isolated after feeding isotopic butyrate or propionate contained an amount of isotopic carbon significantly in excess of that originating from isotopic carbon dioxide. These results indicate that two or more carbon atoms of the butyric and propionic acid molecules contributed in part to the glycogen carbon, but the results of the experiments with acetate gave no indication of two-carbon unit incorporation into glycogen.

As Buchanan and Hastings[231] emphasize, it is not necessary to postulate over-all conversion of fatty acids to carbohydrate in order to explain the appearance of isotopic carbon in glycogen isolated from rats fed isotopic fatty acids and nonisotopic glucose. If butyric acid, for example, is oxidized by the series of reactions proposed for acetoacetic acid, oxalacetic acid containing isotopic carboxyl carbon would be formed according to the following reactions:[229,233]

$$CH_3CH_2CH_2{}^{11}COOH + 2\,O \rightarrow CH_3{}^{11}COOH + CH_3COOH$$

$$CH_3{}^{11}COOH + HOOCCH_2COCOOH \xrightarrow[\text{reactions}]{\text{several}} 2\,CO_2 +$$
$$HOOCCH_2CO^{11}COOH$$
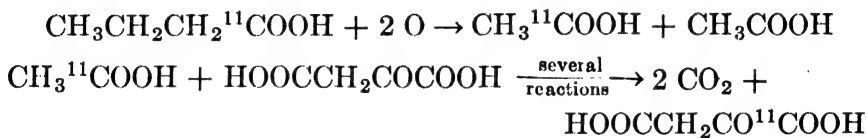
By these reactions, no increase in the total amount of carbohydrate precursors takes place, but the oxalacetate molecules present are renewed, and the newly formed molecules contain isotopic carbon. One mole of nonisotopic oxalacetate is used up for every mole of isotopic oxalacetate formed.

C. PRODUCTION OF GLYCOGEN IN LIVER SLICES

It is known from the experiments of Ostern and associates[234] that liver slices of fasted rabbits will synthesize glycogen in a suitable inorganic medium containing 1% glucose. When labeled bicarbonate was added to the solution, after the lapse of 1 hour 0.17 to 0.45% of the ^{11}C added was found in the glycogen of the liver slices, ^{11}C representing 0.16% and 0.29%, respectively, of the glycogen carbon. These figures obtained by Vennesland et al.[235] represent minimum values.

[233] A. E. Braunstein and M. G. Kritzman, *Enzymologia*, **2**, 129 (1937).

[234] P. Ostern, D. Herbert, and E. Holmes, *Biochem. J.*, **33**, 1858 (1939).

[235] B. Vennesland, *Recent Advances of Medical Physics*. Academic Press, 1948.

When pyruvate was used as substrate, appreciably higher percentages, *i.e.*, 12% of the glycogen so formed, were found to be derived from radioactive carbon dioxide added, in the form of sodium bicarbonate. This is the same order of magnitude as was found for the incorporation of carbon in liver glycogen formed by rats *in vivo*. In these experiments, the incubation was carried out at 38° for 2 hours.[236]

In experiments in which nonisotopic pyruvate was fed to rats and $NaH^{11}CO_3$ was injected, glycogen was formed *in vivo* which contained 14% carbon originating from carbon dioxide.

Thus the results obtained with pyruvate *in vivo* are in agreement with those obtained *in vitro*, while disagreement is observed in the case of glucose.

On the basis of the above theory of fatty acid oxidation, Buchanan and Hastings distinguish between two types of "carbohydrate"-forming substances. The first group consists of immediate carbohydrate precursors (*e.g.*, lactic acid) and substances which are readily converted into these precursors (*e.g.*, propionic acid). These materials upon being fed in sufficient quantity eventually result in an increase in the carbohydrate content of the animal's body and, consequently, cause glycogen formation to take place. The second group, represented by butyrate or acetoacetate, may be converted into carbohydrate precursors through a condensation reaction with oxalacetic acid. An over-all increase in carbohydrate does not result, however, from this reaction. According to the above authors, it would seem, therefore, that the appearance of butyrate carbon in glycogen does not constitute the conversion of fat into carbohydrate in the usually accepted sense, but is a consequence of a renewal of the glycogen molecules present in which the butyrate carbon participates. Evans and Slotin[237,238] incubated mammalian liver slices in labeled bicarbonate–Ringer solution. They found that the bicarbonate in the medium was used in the synthesis of urea.

D. CARBON DIOXIDE FIXATION IN CELL-FREE EXTRACTS OF PIGEON LIVER

Carbon dioxide assimilation in cell-free extracts of pigeon liver was demonstrated by Evans and associates,[239] by making use of $^{11}CO_2$ as

[236] J. M. Buchanan, A. B. Hastings, and F. B. Nesbett, *J. Biol. Chem.*, **145**, 715 (1942).

[237] E. A. Evans, Jr., and L. Slotin, *J. Biol. Chem.*, **136**, 301 (1940).

[238] E. A. Evans, Jr., and L. Slotin, *J. Biol. Chem.*, **141**, 439 (1941). E. A. Evans, Jr., L. Slotin, and B. Vennesland, *ibid.*, **143**, 565 (1942). E. A. Evans, Jr., and L. Slotin, *ibid.*, **136**, 301, 805 (1940).

a tracer, supplying the first proof of carbon dioxide fixation in the animal tissue. The cell-free extracts were found to assimilate 10 to 50% of the labeled carbon dioxide added. Carbon dioxide utilization was measured by incubating a given preparation at 40° with various additions in the presence of a known amount of $^{11}CO_2$. At the end of

Fig. 73. Demonstration of a heat-labile enzyme that decarboxylates oxalacetic acid.[239] Each vessel contained 0.5 ml. 0.1 M acetate buffer, pH 5.0, and water to make a final volume of 2.0 ml. Manganese was added as 0.1 ml. of 0.01 M MnCl₂; the enzyme was 0.5 ml. dialyzed acetone powder extract of pigeon liver; 0.1 ml. oxalacetic acid was tipped from the side arm at zero time after equilibration at 25° C. The heated enzyme was held in a boiling water bath one minute.
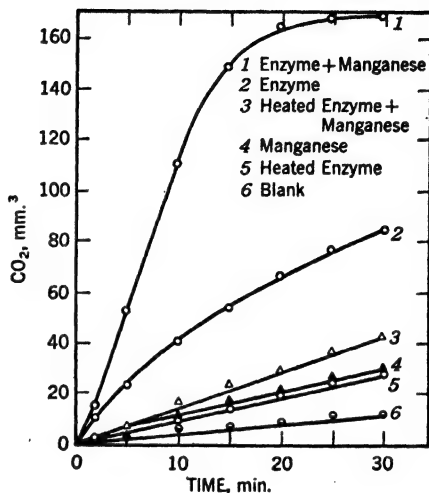
the reaction time, the mixture was deproteinized with a measured amount of 10% metaphosphoric acid and filtered. $^{11}CO_2$ was removed from the filtrate by bubbling carbon dioxide through the solution for 30 minutes. The removal of unwanted labeled compounds by an excess of the corresponding nonlabeled compound is a most useful and often applied procedure in work with isotopic indicators.

The cell-free pigeon liver extracts used contain a heat-sensitive enzyme, activated by manganese ions, which catalyzes the decarboxylation of oxalacetic acid, as seen in Figure 73. The same enzyme is probably involved in the carboxylation itself. In contrast to the oxalacetate decarboxylase of bacterial origin, magnesium ions did not activate the enzyme prepared from pigeon liver. The initial fixation and the subsequent distribution of labeled carbon were found to be the result of the occurrence of a series of reversible reactions.

Evans *et al.* demonstrated carboxylation of pyruvate in a system uncomplicated by the more controversial phases of the Krebs cycle (*cf.* Wood[240]). The initial reaction involved in carbon dioxide fixation by

[239] E. A. Evans, Jr., B. Vennesland, and L. Slotin, *J. Biol. Chem.*, **147**, 771 (1943).
[240] H. G. Wood, *Physiol. Revs.*, **26**, 198 (1946).

pigeon liver was found to be the Wood-Werkman reaction, *i.e.*, the condensation of carbon dioxide and pyruvic acid to oxalacetic acid.[240,241] These authors postulate that carboxyl isotopic pyruvate is formed by a series of reactions involving carboxylation of pyruvic acid to form carboxyl isotopic oxalacetate with distribution of the isotope between both carboxyl groups and decarboxylation of this dicarboxylic acid to pyruvate. Practically all of the carbon dioxide assimilated was present in the pyruvic acid isolated, according to their experiments. When fumarate or malate as well as pyruvate was added to the enzyme preparation in the presence of radioactive bicarbonate, a considerably greater amount of radioactivity was fixed than when pyruvate alone was the substrate. For every mole of fumarate or malate that disappeared, one mole of carbon dioxide was produced and one mole of lactic acid was formed. Only 15% of the total radioactivity assimilated was present in the pyruvate isolated. The remainder was found in the mother liquor and, in view of the absence of other intermediates, was considered to be present in lactate, malate, and fumarate molecules. Furthermore, the reversibility of the oxidative decarboxylation of isocitric acid has been demonstrated by studying the reaction in extracts of pigeon liver–acetone powder with the aid of $^{14}CO_2$.[241a]

The fixation of carbon dioxide by pigeon liver extracts, with pyruvate and fumarate as substrates, was investigated with carbon dioxide containing ^{13}C as well.[242] The ^{13}C was found exclusively in the carboxyl groups of the pyruvate, lactate, malate, and fumarate, and its concentration was approximately the same in all the carboxyl groups. The discussion of this and other extended applications of ^{13}C as a tracer, however, lies outside the scope of this volume. A survey of the application of both radioactive and stable isotopic carbon in intermediary metabolism is given in a recent publication of Buchanan and Hastings[231] and the problem of the fixation of carbon dioxide is discussed by Wood[240] and by Vennesland.[243]

E. RATE OF RENEWAL OF GLYCOGEN

We can often determine the rate of renewal of organic phosphorus compounds (*i.e.*, the percentage of the molecules formed during the

[241] H. G. Wood, C. H. Werkman, A. Hemingway, and A. O. C. Nier, *J. Biol. Chem.*, **135**, 789 (1940).

[241a] S. Grisolia and B. Vennesland, *J. Biol. Chem.*, **170**, 461 (1947).

[242] H. G. Wood, B. Vennesland, and E. A. Evans, Jr., *ibid.*, **159**, 153 (1945).

[243] B. Vennesland, *Recent Advances of Medical Physics*. Academic Press, 1948.

experiment) by comparing the specific activity (activity per milligram phosphorus) of the organic phosphorus with that of the intercellular inorganic phosphorus. This is made possible in many cases by the fact that inorganic phosphorus or a phosphorus-containing precursor which attains rapid equilibrium with the inorganic phosphorus is incorporated with the newly formed organic phosphorus compounds (see page 256).

A comparison of the amount of labeled phosphorus administered with that present in a phosphorus compound obtained from an organ after administration does not permit the calculation of renewal rate, since the administered labeled phosphate is "diluted" by nonradioactive phosphate present in the organism. Nor can we calculate the percentage of liver glycogen which was formed during the experiment by comparing the amount of radiocarbon of the lactate or another labeled carbon compound administered with the radiocarbon content of the liver glycogen. This would require the knowledge of the specific activity of the carbon of the precursor of glycogen or that of another carbon compound which attains rapid equilibrium with the precursor; these data, however, are unknown.

Vennesland and associates[224,235] found that when labeled lactic acid was fed to rats the liver glycogen deposited averaged 21% of the fed lactate, but contained only 3.2% of the lactate radioactivity. A possible explanation of this result is that mixing with precursors is responsible for the small incorporation of ^{11}C from ^{11}C lactate as compared with the much greater net increase observed in the liver glycogen. Other explanations may be given as well. As Wood[240] emphasizes, the feeding of a compound could influence a body component in any of the following ways: (a) the compound fed could be transformed into the body compound and a *net* increase in the component could occur; (b) the compound fed could be transformed into the body component, but *no net* increase in the component would occur; or (c) constituents of the compound administered could be transformed into the body component without the entrance of the compound itself into the body component. In the latter case, the fed compound would not enter the metabolic pool of precursors which serve as a source of carbon for formation of the body component, but rather by indirect action would influence the steady state of the dynamic equilibrium involved in the formation of the body component.

F. ORIGIN OF BICARBONATE OF PANCREATIC JUICE

Ball and associates[244] gave injections of bicarbonate containing radioactive carbon to dogs and found a prompt appearance of the labeled bicarbonate anion in the pancreatic secretion. Its concentration was found to be five times that in the plasma, corresponding to the ratio of the total carbon dioxide concentration in the pancreatic juice to that in the plasma. Under the conditions of these experiments no more than 20% of the pancreatic secretion of bicarbonate could have originated from metabolic carbon dioxide. Strong evidence is thus provided that the bicarbonate contained in the pancreatic juice is derived mainly from the plasma and not from the metabolic carbon dioxide of the gland itself.

G. ELIMINATION OF CARBON MONOXIDE FROM THE HUMAN BODY

The problem of the effect of carbon monoxide upon respiratory metabolism was attacked by Tobias et al.[244a] by making use of labeled ^{11}C ($T = 21$ min.). Several human subjects were given a dose of ^{11}CO via the lungs, followed by 100% oxygen. The exhaled carbon dioxide was collected in soda lime, and measurement for any activity having a half-life of 21 minutes in the soda lime was made. The demonstration of such radioactivity in exhaled carbon dioxide would constitute evidence that carbon monoxide is converted in the human body.

Labeled carbon monoxide formed during the bombardment of a fused boron oxide target in the cyclotron was purified of the simultaneously formed labeled carbon dioxide. Thereafter, it was introduced into a rubber bag containing 150 cc. of ordinary carbon monoxide and 2000 cc. oxygen. The subject, after deep expiration, inhaled and exhaled in the bag three times and, finally, drew the whole contents of the bag into his lungs, holding his breath for 1 minute. This procedure resulted in absorption of at least 95% of the carbon monoxide administered, and the carbon monoxide content of the blood rose correspondingly to two to three volumes per cent. Thereafter, the subject exhaled and breathed air for 7 minutes. He then was given 100% oxygen and all exhaled air was passed through a glass tube containing soda lime sufficient to absorb all exhaled carbon dioxide.

[244] E. G. Ball, H. F. Tucker, A. K. Solomon, and B. Vennesland, *J. Biol. Chem.*, **140**, 119 (1941).

[244a] C. A. Tobias, J. H. Lawrence, F. J. W. Roughton, W. J. Root, and M. I. Gringerson, *Am. J. Physiol.*, **145**, 253 (1945).

Measurement of the radioactivity of the soda lime showed the presence of less than 0.1% of the activity inhaled. From this result it follows that in mild carbon monoxide poisoning only a negligible, if any, proportion of carbon monoxide is burned to carbon dioxide in the body within the first 60 minutes of oxygen breathing. The experiments were limited to 1 hour, owing to the relatively short half-life of the radioactive carbon.

In these experiments, the change of the radioactivity with time in the thigh muscles, the spleen, heart, and liver was studied as well by placing Geiger-Müller counters over these regions. The curves obtained for the muscle and spleen–heart areas were interpreted in terms of the average amount of blood present in them at any given time, and the rate of blood flow.

H. ASSIMILATION OF CARBON DIOXIDE BY YEAST

Ruben and Kamen[245] have found that fresh yeast cells suspended in water assimilated carbon dioxide. In their experiments, approximately one carbon dioxide molecule was reduced for every fifty molecules produced in respiration. Though most of the radioactivity was present in the precipitate formed by the addition of barium ions, the activity did not reside in the carboxyl groups of barium-precipitable material. These results were obtained in extending the work of the above-mentioned investigators with plants, the discussion of which lies outside the scope of this volume.

I. METHANE FORMATION

The formation of methane in the fermentation of ethyl and butyl alcohols by methane-producing bacteria such as *Methanobacterium omelianskii* was interpreted by Van Niel[246] as a process of oxidation by means of carbon dioxide, represented by the general equation:

$$4 H_2A + CO_2 \rightarrow 4 A + CH_4 + 2 H_2O$$
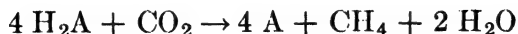
where H_2A is an oxidizable molecule and A is its dehydrogenation product.

A large number of methane fermentations were known in which methane was formed without simultaneous disappearance of carbon

[245] S. Ruben and M. D. Kamen, *Proc. Natl. Acad. Sci. U. S.*, **26**, 418 (1940).

[246] C. B. Van Niel, *Cold Spring Harbor Symposia Quant. Biol.*, **3**, 138 (1935).

dioxide. Tracer techniques were employed by Barker and co-workers[246a] in these reactions to discover whether carbon dioxide is the immediate precursor. Such a reaction is carried out by *Methanobacterium omelianskii* in the fermentation of methyl alcohol according to the equation:

$$4 \; CH_3OH \rightarrow CO_2 + 3 \; CH_4 + 2 \; H_2O$$
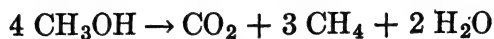
This reaction might be the result of a complete oxidation of four molecules of methyl alcohol to carbon dioxide accompanied by the reduction of three molecules of carbon dioxide to methane. Barker and associates[247] succeeded in showing that methane produced from alcohol in the presence of $^{11}CO_2$ was radioactive.

While, in the formation of acetic acid by the action of *M. omelianskii*, the participation of carbon dioxide in the reaction:

$$2 \; C_2H_5OH + CO_2 \rightarrow 2 \; CH_3COOH + CH_4$$
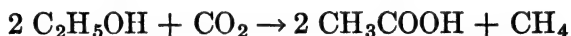
was previously known,[248] it was shown by using $^{11}CO_2$ that the labeled carbon appears in the methane, none being present in the acetic acid. These results strengthen considerably the generalization that methane fermentation of organic as well as of inorganic compounds is essentially an anaerobic oxidation process in which carbon dioxide acts as the ultimate hydrogen acceptor (oxidizing agent). Methane bacteria, like many other living organisms, have thus the ability to convert carbon dioxide into organic constituents of their cells.[249]

J. SYNTHESIS OF CARBON COMPOUNDS BY BACTERIA

Radioactive ^{11}C was employed by Carson et al.[223,250] in the study of carbon dioxide uptake by propionic acid bacteria during the dissimilation of glycerol; similar investigations were carried out using ^{13}C by Wood et al.[241]

If propionic acid originates via succinic acid, as is often assumed, labeled succinic acid supplied to the bacteria in the presence of glycerol and inactive bicarbonate should yield labeled propionic acid through

[246a] H. A. Barker, S. Ruben, and M. D. Kamen, *Proc. Natl. Acad. Sci. U. S.*, **26**, 426 (1940).

[247] H. A. Barker, S. Ruben, and J. V. Beck, *Proc. Natl. Acad. Sci. U. S.*, **26**, 477 (1940).

[248] H. A. Barker, *J. Biol. Chem.*, **137**, 153 (1941).

[249] M. D. Kamen and S. Ruben, *J. Applied Phys.*, **12**, 310 (1941).

[250] S. F. Carson, J. W. Foster, S. Ruben, and M. D. Kamen, *Science*, **92**, 433 (1940).

loss of one of the carboxyl groups. This conclusion was tested in the following way. Radioactive propionic and radioactive succinic acid were added separately to bacterial suspensions in the presence of inactive carbon dioxide and glycerol. The fermentation in the presence of the labeled acids was allowed to proceed for 60 to 90 minutes. No evidence for the reaction succinic acid $\rightleftharpoons$ propionic acid was found under these conditions. From these and further experiments the conclusion was reached that the two main products of the glycerol fermentation, propionic and succinic acids, had been formed by way of each other. An appreciable fraction of the propionic acid was synthesized from $^{11}CO_2$ rather than by a simple transformation of glycerol without degradation of the reduction of the carbon skeleton. Another example is supplied by the above investigation of the reduction of carbon dioxide with an organic compound (in this case glycerol) acting as the ultimate reducing agent.

It has been suggested that the formation of succinate from pyruvate occurs through the following series of reactions:

$$\text{pyruvic acid} + CO_2 \rightleftharpoons \text{oxalacetic acid} \rightleftharpoons \text{malic acid}$$
$$\rightleftharpoons \text{fumaric acid} \rightleftharpoons \text{succinic acid}$$
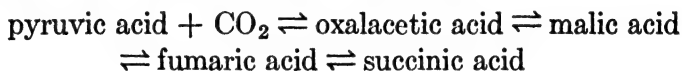
Nishina and associates[251] have succeeded in isolating radioactive crystalline derivatives of malic and fumaric acids from preparations of *Escherichia coli communis* fermenting glycerol and glucose in the presence of radioactive carbon dioxide. They thus furnish additional evidence for the participation of malic and fumaric acids as intermediates in this reaction. When ammonium chloride was present during the fermentation, radioactive aspartic acid could be isolated.

An additional example of the nonphotosynthetic, heterotrophic organisms which are able to reduce carbon dioxide is *Clostridium acidiurici*. The fermentation of purines by *Cl. acidiurici* in the presence of labeled carbon dioxide leads to the formation of acetic acid containing labeled carbon.[246a,247,250]

K. CARBON DIOXIDE UTILIZATION IN THE SYNTHESIS OF ACETIC ACID BY *CLOSTRIDIUM THERMOACETICUM*

Clostridium thermoaceticum catalyzes the reaction:

$$C_6H_{12}O_6 \rightarrow 3\ CH_3COOH$$
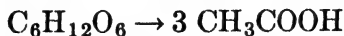
[251] Y. Nishina, S. Endo, and H. Nakayama, *Sci. Papers, Inst. Phys. Chem. Research Tokyo*, **38**, 341 (1941).

Various lines of evidence suggested that carbon dioxide is formed as an intermediate in this reaction. Barker and Kamen[252] found in an early investigation in which ^{14}C was used that the acetic acid produced by this organism fermenting sugar in the presence of $^{14}CO_2$ is radioactive, the radioactive isotope being approximately equally distributed between the methyl and carboxyl groups. The application of the isotope dilution method (see page 91) indicated that two moles of carbon dioxide are formed for each mole of glucose, and that this carbon dioxide is then incorporated into acetic acid.

L. CARBON DIOXIDE UTILIZATION IN THE SYNTHESIS OF ACIDS BY *BUTYRIBACTERIUM RETTGERI*

Butyribacterium rettgeri causes a modified butyric acid type of lactate fermentation, the main products being carbon dioxide and acetic and butyric acids. This fermentation is remarkable because of the low

TABLE 161

Fermentation of Lactate[253] in the Presence of $^{14}CO_2$

Compound	mM per ml.	Counts per min. per mM	Total counts per min.
Lactic acid, decomposed............	0.463	——	——
Carbon dioxide, initial............	0.115	33800	3890
Carbon dioxide, final.............	0.298	4800	1430
Acetic acid.......................	0.252	2470	620
Butyric acid.....................	0.177	6350	1120

yield of carbon dioxide and the high yield of fatty acids. To investigate the possible conversion of carbon dioxide into fatty acids, *B. rettgeri* was allowed to grow in a medium containing lactate and $^{14}CO_2$. Some of the results obtained by Barker and co-workers are in Table 161 (*cf.* Barker and Haas[254]). The carboxyl group of acetic acid was found to contain significantly more ^{14}C than the methyl group. All four positions in butyric acid contain carbon derived from carbon dioxide. This investigation shows that the fermentation of lactate by *B. rettgeri* can be fitted into the following simplified reaction scheme:

[252] H. A. Barker and M. D. Kamen, *Proc. Natl. Acad. Sci. U. S.*, **31**, 219 (1945).

[253] H. A. Barker, M. D. Kamen, and V. Haas, *Proc. Natl. Acad. Sci. U. S.*, **31**, 355 (1945).

[254] H. Barker and V. Haas, *J. Bact.*, **47**, 301 (1944).

$$CH_3CHOHCOOH + H_2O \rightleftharpoons CH_3COOH + CO_2 + 4 H$$
$$2 CO_2 + 8 H \rightleftharpoons CH_3COOH + 2 H_2O$$
$$2 CH_3COOH + 4 H \rightleftharpoons CH_3CH_2CH_2COOH + 2 H_2O$$
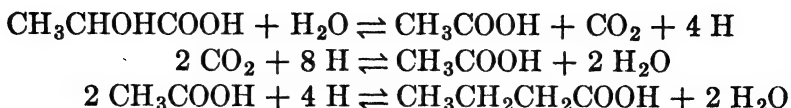
No exchange was found to occur between the ^{14}C of carbon dioxide and acetic or butyric acid. The absence of such an exchange was verified by an experiment in which lactate was fermented in the presence of acetic acid labeled both in the methyl and carboxyl groups. After the fermentation, no ^{14}C could be detected in carbon dioxide, while about 57% of the ^{14}C was present in butyric acid molecules.

M. SYNTHESIS OF BUTYRIC AND CAPROIC ACIDS BY *CLOSTRIDIUM KLUYVERI*

Clostridium kluyveri was found to produce a reaction very similar to the conversion occurring in fatty acid synthesis by animals. *Cl. kluyveri*, growing anaerobically in a medium containing ethanol and synthetic acetic acid labeled in the carboxyl group with ^{14}C, forms labeled butyric and caproic acids.[255] The butyric acid thus formed has ^{14}C almost equally distributed between the carboxyl carbon and the β-carbon atoms. The α- and γ-positions are inactive. In caproate one-third of the ^{14}C is in the carboxyl group, the rest presumably in the β- and Δ-positions. No active carbon dioxide is formed in these experiments from carboxyl-labeled acetic acid. This indicates that carbon dioxide is not an intermediate in these reactions.

The formation of caproic acid from butyric acid and ethyl alcohol was investigated by the use of carboxyl-labeled butyric acid. There were two possibilities for the formation of the C_6 compound from the C_4 and C_2 substrates. The carboxyl carbon of the butyric acid could condense with the methyl carbon of the alcohol, or the carbonyl carbon of the alcohol could condense with the terminal methyl group of the butyric acid. The demonstration that the caproic acid formed contained the isotope only in the β-position proves that only the first of these possibilities is realized.

Numerous compounds containing ^{14}C were synthesized in applied metabolic studies. Among those are[255] butyric acid ($CH_3CH_2CH_2{}^{14}COOH$), caproic acid ($CH_3CH_2CH_2CH_2CH_2{}^{14}COOH$), and propionic acid ($CH_3CH_2{}^{14}COOH$).

[255] H. A. Barker, M. D. Kamen, and B. T. Bornstein, *Proc. Natl. Acad. Sci. U. S.*, **31**, 373 (1946), and *personal communication*.

N. ROLE OF CARBON DIOXIDE IN THE METABOLISM
OF UNICELLULAR ORGANISMS OTHER THAN BACTERIA

The molds *Rhizopus nigricans* and *Aspergillus niger* ferment glucose and sucrose in the presence of $^{11}CO_2$ with the formation of isotopic fumaric and citric acids, respectively.[256]   In both cases, isotopic carbon was found only in the carboxyl group.   The discussion of the application of labeled carbon in the study of photosynthesis lies outside the scope of this volume (*cf.* Rabinowitch[256a]).   It was in a study of photosynthesis that ^{11}C was first applied as a tracer in biological studies by Ruben, Hassid, and Kamen.[257]

O. DISTRIBUTION OF LABELED TYROSINE IN RATS AND MICE

Tyrosine labeled with radioactive carbon, ^{14}C, in the β-position was used by Greenberg *et al.*[258] to investigate the metabolism of this amino acid in normal and tumor-bearing (lymphosarcoma) rats. Following the intravenous injection of this coumpound, ^{14}C was rapidly incorporated into the protein of the various organs of each animal and was retained to a considerable extent after a period of 3 days.   Intestinal mucosa, kidney, and plasma proteins, followed closely by the liver, had the highest ^{14}C concentrations (see Table 162).   The tumor protein also showed high activity, and accounted for one-third to one-fourth of the total incorporated isotope (see Table 163).   Amino acid isolation experiments showed that virtually all the radioactivity of the proteins was due to tyrosine itself.   Very low concentrations of isotope were found in the dicarboxylic amino acids.   Moderate concentrations of ^{14}C were found in tyrosine and in ketones bodies isolated from the urine with the aid of carriers.   Much lower concentrations were found in urinary urea, creatinine, and hippuric acid.   Reid and co-workers[259] injected into the tail vein of three mice bearing melanosarcoma tyrosine labeled in the β-position.   Each mouse received 0.9 mg. tyrosine of an activity of 0.8 microcurie (about 11,700 counts per minute).

[256] J. W. Foster, S. F. Carson, S. Ruben, and M. D. Kamen, *Proc. Natl. Acad. Sci. U. S.*, **27**, 590 (1940).

[256a] E. Rabinowitch, *Photosynthesis and Related Processes.* Interscience, New York, 1945.

[257] S. Ruben, W. Z. Hassid, and M. D. Kamen, *J. Am. Chem. Soc.*, **61**, 661 (1939).

[258] D. M. Greenberg, *personal comm nication.*

[259] G. C. Reid *et al.*, *personal communication.*

Table 164 shows the activity of the tissues in counts per minute per milligram dry tissue.   Thyroid, adrenals, tumor, kidneys, and intestines show the largest uptake of ^{14}C.

TABLE 162

Distribution of ^{14}C of Isotopic Tyrosine in Tissue
Protein of the Normal Rat[258]

Tissue[a]	Relative concentration per mg. protein	Concentration per mg. tyrosine, per cent foosde	Total ^{14}C in tyrosine of tissue, per cent of dose
Intestinal mucosa....	100	0.28	2.1
Kidney..............	71	0.12	1.3
Blood plasma.......	53	0.10	0.6
Liver...............	31	0.084	3.7
Carcass.............	9	0.030	25.0
Testes..............	16	0.028	0.2
Brain..............		0.024	0.2
Muscle.............	6	0.017	1.1
Red blood cells......	1		
Total..........			*34.2*

[a] ^{14}C in urine = 28.5%; ^{14}C expired as CO_2 = 15%.

TABLE 163

Distribution of ^{14}C of Isotopic Tyrosine in Tissue
Protein of the Tumor Rat[258]

Tissue[a]	Relative concentration per mg. protein	Concentration per mg. tyrosine, per cent of dose	Total ^{14}C in tyrosine of tissue, per cent of dose
Intestinal mucosa....	100	0.175	0.81
Kidney.............	62	0.106	1.05
Blood plasma.......	59	0.110	0.64
Tumor[b]............	*55*	*0.112*	*10.75*
Liver...............	26	0.052	3.45
Carcass.............	2	0.024	20.8
Testes.............	12	0.021	0.16
Brain..............	7	0.013	0.07
Muscle.............	1	0.009	0.36
Total..........			*38.1*

[a] ^{14}C in urine = 15.5%.
[b] Tumor contained 28% of incorporated tyrosine although it comprised only 9% of body weight.

TABLE 164

[14]C Content of Tissues of Mice after Administration of Labeled Tyrosine[259]

Tissue	Activity per mg. dry tissue		
	0.5 hr.	24 hr.	72 hr.
Adrenals......................	29.0		15.0
Bone........................	4.05		2.22
Brain.......................	7.13		
Eyes........................	5.6	4.0	2.7
Pancreas and fat pad.............	11.8	2.9	
Omental fat....................		5.74	
Gall bladder and contents........	ca. 3.0	ca. 8.0	ca. 9.0
Heart.......................	16.6	13.5	5.7
Intestines....................	31.9	22.4	10.1
Intestinal contents_a_.............	31.9	26.2	7.4
Kidneys......................	28.6		14.9
Lungs.......................	13.4		
Liver........................	20.4	14.6	11.3
Lymph nodes..................	14.9	9.74	
Muscle.......................	5.57	3.86	4.80
Plasma.......................	44.0		
Red corpuscles.................	1.27		
Skin........................	11.1	7.71	
Spleen.......................	17.7	14.5	9.63
Stomach and contents............	16.0	16.8	11.2
Testes.......................	9.96	7.16	
Thyroids.....................	ca. 39.0	ca. 14.0	ca. 57.0
Tumor.......................	24.4	19.9	16.5
Urine_b_.....................	60.9	54.1	
Pancreas.....................			6.63

_a_ Samples removed from intestines at dissection.
_b_ Samples taken from bladder at dissection.

P. PRECURSOR OF ADRENALINE

In a recent study on the precursor of adrenaline, Gurin and Delluva[260] used _dl_-phenylalanine labeled with ^{14}C in the carboxyl or α-carbons. The phenylalanine was synthesized from doubly labeled glycine. Upon administration to rats, this substance was converted to radioactive adrenaline which could be recovered by the addition of nonradioactive adrenaline as a carrier to extracts of the adrenals. The recovered adrenaline was radioactive and found to contain ^{14}C localized in the terminal carbon of the side chain.

[260] S. Gurin and A. M. Delluva, _J. Biol. Chem._, **170**, 545 (1947).

Tritium-labeled phenylalanine was also applied in this investigation. It was also found to be converted to adrenaline. The results obtained suggest that the biological conversion involves decarboxylation of phenylalanine or one of its derivatives and that the resulting aminoethyl side chain remains attached to the benzene nucleus during the biological synthesis of adrenaline.

The radioactivity of the phenylalanine and that of the recovered adrenaline is demonstrated by the data in Table 164A.

TABLE 164A

Results of Administration of Tritium-Labeled and ^{14}C-Labeled Phenylalanine[260]

Tritium experiment (^{3}H, counts per mole per min.)		^{14}C experiments[a] (^{14}C, counts per mole C per min.)	
Administered tritio-phenylalanine..........	7×10^6	Administered phenylalanine (α-carbon)	4.4×10^6
Body water (blood).......	2×10^5	Recovered adrenaline....	3.0×10^3
	1.2×10^4	Formic acid C from adrenaline..........	2.2×10^4

[a] Average of 3 experiments.

One of the outstanding results of the hitherto restricted application of labeled carbon in metabolic studies is that carbon dioxide may no longer be regarded simply as an end product of metabolism, but must be thought of as an essential compound in certain metabolic reactions not only in lower, but also in higher forms of life. Important information was, furthermore, obtained concerning the reactions relating carbohydrate and fat precursors and their contribution to the metabolic pool of each of the precursors of adrenaline, etc. Besides leading to novel results, the application of labeled carbon greatly promoted the interpretation of information obtained by usual analytical methods. While ^{32}P has been the most applied radioactive indicator, in the future ^{14}C may outdistance radiophosphorus.

Path of Intermediary Reactions

I. Application of Labeled Phosphate in the Study of Intermediary Reactions in Glycolysis

Experiments in cell-free tissue extracts were carried out in order to study the intermediary reactions of glycolysis.[1,2] The following equations show the way in which inorganic phosphate participates in the splitting of sugar:

(a) 1 glyceraldehydephosphoric acid + 1 cozymase + 1 adenosine-diphosphoric acid + 1 phosphoric acid ⇌ 1 phosphoglyceric acid + 1 dihydrocozymase + 1 adenosinetriphosphoric acid; or, alternately,

(b) 2 glyceraldehydephosphoric acid + 2 cozymase + 1 adenosine-monophosphoric acid (adenylic acid) + 2 phosphoric acid ⇌ 2 phosphoglyceric acid + 2 dihydrocozymase + 1 adenosinetriphosphoric acid.

When equilibrium is attained, the application of the usual chemical methods does not indicate any changes taking place in the above-mentioned systems. However, by labeling the phosphate with ^{32}P, we can observe a gradual replacement of active phosphate ions by non-active ones, and vice versa. The application of the radioactive indicators permits one to split the dynamic equilibrium into its two components and to measure the velocity of each of the reactions.

Meyerhof and associates,[1] combined reaction b with the reoxidation reaction of dihydrocozymase:

(c) 2 dihydrocozymase + 2 pyruvic acid ⇌ 2 cozymase + 2 lactic acid, investigating thus the reaction:

(d) 2 triosephosphoric acid + 2 pyruvic acid + 1 adenosinemono-phosphoric acid + 2 phosphoric acid ⇌ 2 phosphoglyceric acid + 2 lactic acid + 1 adenosinetriphosphoric acid. The cozymase acts as a catalyst.

[1] O. Meyerhof, P. Ohlmeyer, W. Gentner, and H. Maier-Leibnitz, *Biochem. Z.*, **298**, 396 (1938).

[2] J. K. Parnas, *Enzymologia*, **5**, 168 (1938); *Bull. soc. chim. biol.*, **21**, 1059 (1939). G. Hevesy, T. Baranowsky, A. J. Guthke, P. Ostern, and J. K. Parnas, *Acta Biol. Exptl. Warsaw*, **12**, 34 (1938).

A. EFFECT OF COZYMASE ON THE INTERCHANGE BETWEEN INORGANIC AND CREATINE PHOSPHATE

No significant interchange takes place between creatine P and inorganic P in a muscle extract dialyzed for 50 hours in absence of cozymase. If, however, cozymase is added to the system, an appreciable percentage of creatine P interchanges with inorganic P as seen in Table 164B.

TABLE 164B

Effect of Cozymase on P Interchange between Creatinephosphoric Acid and Creatine Phosphate[1]

System	Labeled inorganic P, mg.		Creatine-phosphoric acid P, mg.	Labeled creatine-phosphoric acid P, mg.
	After 0 min.	After 30 min.	After 30 min.	After 30 min.
Muscle extract dialyzed 6 hr...............	1.15	0.810	0.65	0.315
Muscle extract dialyzed 50 hr. without cozymase...............	1.29	1.25	1.09	0.03
Muscle extract dialyzed 50 hr. with 0.4 mg. cozymase..........	1.29	0.91	1.15	0.40

In these experiments, 6 mg. creatine, 0.1 mg. Mg, and adenosine triphosphate containing 0.43 mg. labile P were added to 1 ml. muscle extract. The total volume amounted to 2.1 ml.; the experiments were carried out at 40° C. in the presence of 1 mg. phosphopyruvic acid.

B. TRANSFER OF PHOSPHATE GROUPS

A phosphate group may be transferred from one organic molecule to another without passing through the inorganic stage; this can be shown in several cases by an absence of ^{32}P in the reaction products in spite of the presence of labeled inorganic phosphate (see also Hevesy et al.[2]). If phosphoglyceric acid, magnesium, and labeled inorganic phosphate are added to muscle extract, the reversible reaction:

$$\text{3-phosphoglyceric acid} \rightleftharpoons \text{2-phosphoglyceric acid}$$
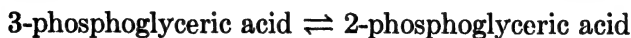
was found to take place without incorporating active phosphate in the phosphoglyceric acid. In a similar way, the transformation of glucose-1-phosphoric acid (Cori ester) into glucose-6-phosphoric acid (Robison

ester) was studied by Parnas and co-workers[2] in the presence of labeled inorganic phosphate; it was found that the esters did not become active. Furthermore, when adenosinetriphosphoric acid was formed from adenylic acid and phosphoglyceric acid in the presence of labeled phosphate, the phosphoglyceric acid was found to be inactive. In all these cases, a direct transfer of phosphate groups from one molecule to another took place without passing through the inorganic stage. A possible explanation of the above results is that, for example, phosphoglyceric acid and adenylic acid form a molecular complex with the enzyme, the phosphate radical is shifted from one molecule to the other, and then the products, phosphoglyceric acid and adenosine triphosphate, leave the enzyme surface. The labeled inorganic phosphate present in the solution then clearly has no opportunity to participate in the reactions.

When adenylic acid is formed from adenosine in the presence of hexose diphosphate and labeled inorganic phosphate in yeast, one-half the phosphorus atoms were found to be those originally located in the hexose diphosphate molecules, while the other half were originally present as inorganic phosphate.[2]

C. EXCHANGEABILITY OF COZYMASE PHOSPHATE

If the cozymase molecule splits off phosphate in the course of the above-mentioned reaction and is rebuilt, incorporating other phosphate radicals, the cozymase molecule in the presence of labeled phosphate · should become active. To test this point, 300 mg. B-protein, 40 mg. cozymase, 10.5 mg. phosphorus as hexose diphosphate, 40 mg. acetaldehyde, 10.5 mg. labeled phosphorus as inorganic phosphate, 90 mg. glucose, 0.5 mg. magnesium, and 0.15 mg. manganese were mixed. The mixture was $1/60$ M in sodium fluoride, and $1/130$ M in ammonium carbonate. At 28° C., in the course of 75 minutes, 5800 ml. carbon dioxide was produced, corresponding to the oxidation of 7.8 mg. hexose diphosphate. From the above figures it follows that each cozymase molecule participated in the interchange cozymase $\rightleftharpoons$ dihydrocozymase 4.5 times during the experiment. In spite of this fact, the cozymase isolated after the lapse of 75 minutes was found to be entirely inactive.[1] In yeast, some interchange of the phosphate group of cozymase with labeled inorganic phosphate was recently reported (see page 346).

D. CONVERSION OF β-GLYCEROPHOSPHORIC ACID INTO THE α FORM

Radiophosphorus was employed by Chargaff[3] to investigate whether the migration of phosphoric acid from the β to the α position of glycerol, which can be effected by acid catalysis or by enzymes, is accompanied by exchange between the esterified phosphoric acid and the inorganic phosphates of the surrounding medium. To a solution of sodium β-glycerophosphate, labeled sodium phosphate and concentrated sulfuric acid were added. The barium α-glycerophosphate obtained after barium hydroxide was added to the solution, which had been kept boiling, was found to be inactive. The same negative result was obtained when the hydrolysis of β-glycerophosphoric acid was effected by kidney phosphatase in the presence of labeled sodium phosphate. Thus during isomerization of β-glycerophosphoric acid to the α form in acid medium the esterified phosphoric acid could at no time have been in equilibrium with the inorganic phosphate of the solution. In both reactions studied, the radioactive phosphate of the medium failed to exchange with the organic phosphoric acid ester.

The absence of radioactivity from the residual glycerophosphoric acid isolated in enzyme experiments which were stopped before hydrolysis was complete demonstrates that under the experimental conditions no appreciable synthesis of the phosphoric acid ester from glycerol and inorganic phosphate took place and that the enzymic attack did not labilize the substrate to such an extent as to induce free exchange with the inorganic phosphate of the medium.

II. Conversion of Prothrombin to Thrombin

It has long been a matter of conjecture whether thrombin is a reaction product of prothrombin, calcium, and the thromboplastic factor (or the cephalin contained in it), or whether these substances interacted in some different manner. Labeled phosphate was used to answer this question.[4] Radioactive thromboplastin was isolated from rats given labeled phosphate. Experiments carried out making use of the radioactive thromboplastin showed that the amount of phosphorus-containing compounds transferred to the thrombin, if at all significant, is extremely small.

[3] E. Chargaff, *J. Biol. Chem.*, **144**, 455 (1942.)
[4] E. Chargaff, M. Ziff, and S. S. Cohn, *J. Biol. Chem.*, **135**, 351 (1940).

III. Exchange between Inorganic and Acetyl-Bound Phosphate

By use of radioactive phosphate, the exchange between inorganic and acetyl-bound phosphate in extracts of *Clostridium butylicum* and of *Escherichia coli* was studied by Lipmann and Tuttle.[5] Reversibility of a reaction of the phosphoroclastic type:[6]

$$CH_3COCOOH + HOPO_3 \rightleftharpoons CH_3COO{\sim}PO_3 \ (H_2 + CO_2) \text{ or } HCOOH$$
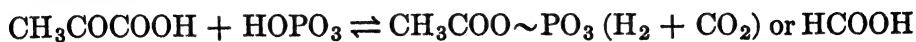
involves a continuous shift between inorganic and acetyl phosphate; this exchange was explored with labeled phosphate. The general procedure of these experiments was to place in the main part of a Warburg vessel the enzyme with all additions, except acetyl phosphate, and in the side arm a mixture of acetyl and radioactive inorganic phosphate. The vessel was then filled with the desired gas mixture, brought to temperature equilibrium, and the experiment was started by dipping the contents of the side arm into the main part of the vessel. The experiment was terminated by addition of trichloroacetic acid to the cooled vessel. The degree of turnover is estimated as exchange percentage

TABLE 165

Exchange of Radioactive Phosphorus in Extracts of *Clostridium butylicum*[5]

Expt. No.[a]	Incubation time, min.	Gas phase (+18% CO_2)	P^*_{ac}, mμc.	P^*_i, mμc.	P^*_{ac}, %	P^{chem}_{ac}, %	Exchange, %
1	3	H_2	31.5	114.5	21.5	64[b]	34
		N_2	30.5	113	21	62	34
	10	H_2	36	121	23	57	40
		N_2	33	115	22	59	37
2	0		9	510	2	37	(5)
	28	H_2	133	410	25	24[c]	104
			130	400	24	31	77
		N_2	135	410	25	32	78

[a] Experiment 1: 0.3 ml. 10% enzyme solution in 0.8 ml. water, 37°; experiment 2: 0.4 ml. 10% enzyme solution in 0.9 ml. water, 37°; plus additions as indicated in the table.

[b] $P^{chem}_{ac} + P^{chem}_i$ is 1.49 mg. phosphorus.

[c] $P^{chem}_{ac} + P^{chem}_i$ is 1.10 mg. phosphorus. The degree of exchange is then expressed by the following relation:

$$\text{Exchange } (\%) = \frac{P^*_{ac}/(P^*_{ac} + P^*_i)}{P^{chem}_{ac}/(P^{chem}_{ac} + P^{chem}_i)} \times 100$$

[5] F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 505 (1945).

[6] The sign $\sim$ is used by Lipmann and associates to distinguish the energy-rich phosphate bond with an average bond energy of 12 kg.-cal. from the ordinary ester bond with about 3 kg.-cal.

from chemical (P^{chem}) and radioactivity (P^*) determinations on the acetyl phosphate (P_{ac}^{chem}, P_{ac}^*) and inorganic (P_i^{chem}, P_i^*) phosphate fractions, respectively.

As seen in Table 165, after the lapse of 3 minutes a 34% exchange is reached; in analogous experiments with *Escherichia coli* an exchange of 30% is observed after the lapse of 20 minutes. The rate of exchange is little or not at all influenced by the addition of reactants other than acetyl phosphate.

IV. Application of Labeled Sulfur

A. ORIGIN OF HYDROGEN SULFIDE PRODUCED BY ACTION OF SULFUR ON THIOLS

When thiol compounds such as cysteine, gluthathione, and proteins are treated with sulfur, hydrogen sulfide is produced. To determine if the hydrogen sulfide formed in the reaction originates from the elementary sulfur used, the following experiment was carried out. With an excess of cysteine in a phosphate buffer, the labeled sulfur in part of the solution was reduced. The activity of the hydrogen sulfide collected and that of the remaining cystine–cysteine was then investigated. No activity was found in the cystine–cysteine, while the radioactivity of the hydrogen sulfide evolved accounted for most of that added.[7]

B. ENZYMIC FORMATION OF CYSTEINE

In investigations in which cysteine and hydrogen sulfide containing radioactive sulfur have been added to an enzyme preparation which converts cysteine to pyruvic acid, ammonia, and hydrogen sulfide, the cysteine was found to contain an appreciable amount of radioactive sulfur, as is seen in Table 166. This result demonstrates the formation of cysteine sulfur from sulfide sulfur under these conditions.[8]

TABLE 166·

Sulfur Exchange between Cysteine and Radioactive Sulfur[8]

Cysteine added, mg.	Labeled H_2S added, mg.	Activity of labeled H_2S added, counts/min.	Time, hr.	Cysteine isolated, mg.	Activity of cysteine, counts/min
76	1.53	2,700,000	3.00	7.5	1890
153	3.06	5,400,000	3.00	40.0	398
192	1.70	3,060,000	2.25	30.9	560

[7] H. Tarver and C. L. A. Schmidt, *J. Biol. Chem.*, **146**, 69 (1942).

[8] C. V. Smythe and D. Halliday, *J. Biol. Chem.*, **144**, 237 (1942)

Skeleton Metabolism

I. General Remarks

Bone formed in an organism containing labeled phosphate must become labeled, since the ultimate source of the bone phosphate is the circulating phosphate. By comparing the radioactivity of the mineral bone phosphate of the growing skeleton with the radioactivity of the inorganic plasma phosphate we are able to get information on the extent of mineralization which took place in the skeleton since the administration of labeled phosphate.

From the classical work of Robison[1] we know that organic phosphorus compounds and phosphatase play an important part in the calcification process. In a more recent work, Gutman et al.[2] have shown that phlorizin and phloretin, which inhibit glycogenolysis, also stop in vitro calcification. This they found from the fact that arrested calcification could be restored by addition of a phosphoric ester such as glucose-1-phosphate and these authors concluded that phlorizin and phloretin act on calcification by inhibiting the processes of phosphorylative glycogenolysis essential for in vitro calcification of cartilage in solutions containing phosphorus in the form of inorganic phosphate only.

Comparison of the activity of ^{32}P of bone mineral phosphate with the activity of inorganic plasma phosphate should give a correct estimate of the renewal of bone apatite if the phosphate incorporated in bone tissue is derived directly from the inorganic plasma phosphate or from an organic phosphate compound in rapid equilibrium with it. The rate of renewal of the molecules of many of the phosphoric esters present in the animal organism is known to be a rapid process; consequently, the above assumption on the determination of the extent of renewal by comparison of the specific activity of bone mineral phosphate with the specific activity of inorganic plasma phosphate will be justified.

[1] R. Robison, *The Significance of Phosphorus Esters in Metabolism*, 1912.
[2] A. B. Gutman, F. B. Warrick, and E. B. Gutman, *Science*, **95**, 461 (1942).

Roche and Deltour[3] compared the increase in calcium and phosphorus content of cartilage and bone metaphyses of the sheep *in vitro*, once when phosphate and calcium were added to the solution containing the tissue, then after addition of an equivalent amount of glycerophosphate and calcium. In the last-mentioned case, calcification was found to be more pronounced. These authors interpret their results by assuming that the formation of calcium phosphate is preceded by a combination of phosphate ions split off under the action of bone enzyme and the protein present in bone tissue.

No significant difference is found in the rate of uptake by the skeleton of ^{32}P administered as sodium phosphate and as calcium phosphorylcholine chloride.[4]

II. Distribution of Labeled Phosphorus in the Incisors of the Rat

The rapidly growing incisors of the rat are well suited for the study of the distribution of phosphorus in growing bone tissue. The average incisor growth per week amounts to 2.7 mm. in adult, and to 3.4 mm. in young rats. The cross section of the pulp is very large at the proximal end, becoming narrower toward the distal end, and the last few millimeters of the tooth are free of pulp.

After cutting the incisors transversely into pieces and analyzing them separately, it was observed that the larger part of the labeled phosphate is found in those regions of the incisor in which the pulp is wide, but that some labeled phosphate is present in every part of the incisor tissue. This finding becomes clear from Table 167.

Although the figures in the table show that the deposition of labeled phosphorus is not restricted to the regions in the vicinity of the pulp, the labeled phosphorus being found even in the most remote part of the incisor, an attempt was made to obtain incisors with an appreciably larger pulp-free part. As is well known, rats, being rodents, grind their teeth and, thus, continuously remove parts of the pulp-free end of the growing incisors. By extracting the upper incisors the animal was prevented from gnawing and, consequently, incisors could be obtained with a pulp-free distal end 10.5 mm. long. The results of this experiment are shown in Figure 74. In the experiment the content of labeled phosphorus was found to vary between 0.01% at the proximal end and

[3] J. Roche and G. Deltour, *Compt. rend.*, **216**, 748 (1943).

[4] R. F. Riley, B. McCleary, and R. E. Johnson, *Am. J. Physiol.*, **143**, 677 (1945).

0.000033% at the pulp-free distal end, thus diminishing by a factor of 1/300. Figure 74 shows both the location of the pulp and the distribution of the labeled phosphorus. The lower set of values in the figure

TABLE 167

Distribution of Labeled Phosphorus, Given in a Normal Diet, in the Incisors of a Rat[a] after Two Days[5]

| Part of incisor[b] | Ash wt., mg. | Per cent labeled P | |
		Found	Per mg. ash
Proximal 1 u	38.2	0.42	0.011
Proximal 2 u	40.8	0.47	0.012
Proximal 1 l	29.2	0.37	0.013
Proximal 2 l	27.2	0.38	0.014
Middle	92.6	0.125	0.00135
Distal 1 u	115.2	0.072	0.00063
Distal 2 l	36.4	0.008	0.00022

[a] Rat weight = 210 g. Labeled P found in total incisors = 1.85%; average per milligram ash = 0.005%. Greatest ratio between proximal and distal end = 60.

[b] u denotes upper; l denotes lower.

give the relative amounts of labeled phosphorus per milligram fresh tissue in the corresponding section. The figures above give the length of the section in millimeters. It is clearly seen that the bulk of the
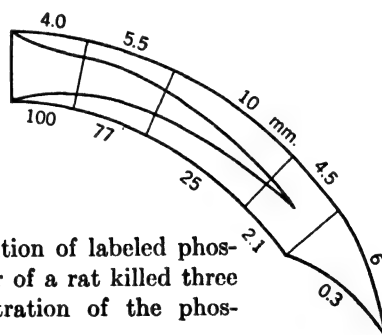
Fig. 74. Distribution of labeled phosphorus in the incisor of a rat killed three days after administration of the phosphorus.[5]

labeled phosphorus is found in the vicinity of the pulp, but that a far from negligible amount reaches even the remotest part of the incisor.

[5] G. Hevesy, J. J. Holst, and A. Krogh, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **13**, 13 (1937).

One part of the first section of the incisor shown, *i.e.*, 1.4 mm., grew during the time which elapsed between the injection of the labeled phosphorus and the killing of the animal; the remaining parts were present before the labeled phosphorus was given. Of 204 parts of labeled phosphorus, only 100 were found in the first section and, consequently, no more than 30 were present in the part actually grown, while the remaining 174 or more were, at least partly, located in the parts present before the injection of phosphorus.

When seeking an explanation for the presence of labeled phosphorus at a considerable distance from the pulp, it should be kept in mind that even the most remote incisal part of the tooth contains organic constituents and water. The constituents of the blood plasma penetrate the latter, and an exchange of phosphate radicals and possibly also some ossification occurs *in situ*, although only to a slight extent, owing to the poor circulation relative to that in the vicinity of the pulp.
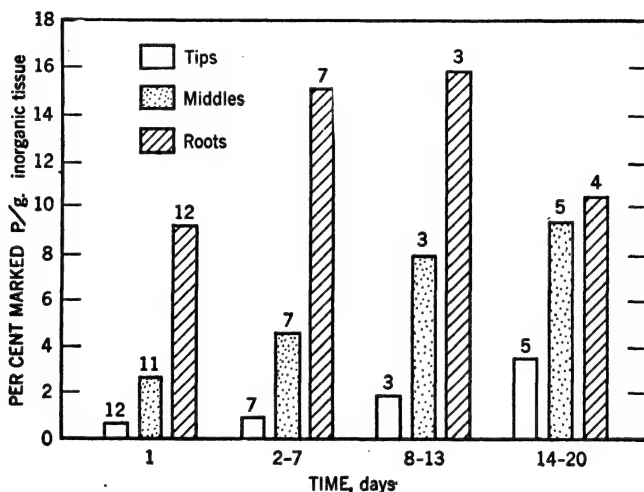
Fig. 75. Retention of marked phosphorus fed as Na HPO$_4$ by the tip, middle, and root portions of the incisors of rats.[6] The figure above each column is the number of animals represented by the average.

The partition of the labeled phosphorus in the incisor of the rat at different times clearly demonstrates the effect of the growth; this appears in Figure 75, which is taken from a paper by Manly and Bale.[6]

[6] M. Manly and W. F. Bale ,*J. Biol. Chem.*, **129**, 125 (1939).

III. Uptake of Labeled Phosphorus by Organs of Rats Raised on a Phosphorus-Deficient Diet

One group of rats was fed from weaning a diet containing 0.30% each of calcium and phosphorus. Another group was fed a diet containing only 0.12% of one of these elements. When the animals were 49 days old, they were injected subcutaneously with a solution containing ^{32}P; they were allowed to live for another 90 hours, the same diets being continued. The animals on the diet low in calcium and phosphorus retained, on an average, 91.1% of the injected ^{32}P, while those on the diet high in calcium and phosphorus retained 73%. The bulk of the extra radiophosphorus retained by the animals on the poorer diet was found in the muscles.[7]

IV. Variation of Mineral Content of the Skeleton with Age

Labeled phosphate so far has not been applied as much in the study of the formation of growing bone as in the investigation of rate of renewal of the fully grown skeleton. The latter problem can obviously be solved only by making use of an isotopic indicator. In an attempt to elucidate this problem it should be realized that, while scarcely any increase in weight of the rat is observed, very marked additional mineralization of bone tissue may take place. This was clearly shown by Roche and collaborators,[8] who, as a measure of the extent of mineralization of

TABLE 168

Phosphorus : Nitrogen Ratio of Rat Bones[5]

Bone	P : N for animals weighing		
	100–120 g.	150–170 g.	200–210 g.
Femur................	1.2	3.1	2.4
Tibia.................	1.5	3.0	2.3
Skull.................	1.9	2.6	1.6
Incisor..............	4.3	4.9	3.3

bone tissue, considered the ratio of its phosphorus and nitrogen contents. In Table 168 are computed the average protein nitrogen and phosphorus contents of bone tissue of rats weighing 100–210 g.; the data, which assume the protein to contain 16% nitrogen, are taken from the work of these investigators.

[7] W. E. Gaunt, H. D. Griffith, and J. T. Irving, *J. Physiol.*, **100**, 372 (1942).

[8] A. Roche and J. Garcia, *Bull. soc. chim. biol.*, **18**, 1014 (1936).

In early growth, the rate of mineralization lags somewhat behind the rate of growth, the phosphorus : nitrogen ratio diminishing with increasing growth. As soon as a weight of 30–40 g. is reached, the rate of mineralization increases more rapidly than the body weight, until a weight of 110–120 g. is attained. In the subsequent phase of growth, the weight of the rat increases only moderately, while a marked additional mineralization of the bone tissue takes place. When the weight of the rat reaches 160 g., the period of "old age" sets in and, from this moment, a gradual loss of mineral constituents and a corresponding decrease in the ratio of phosphorus to nitrogen can be observed.

After the administration of ^{32}P to a rat weighing 120 g., additional mineralization will take place and, in a labeled medium, the ^{32}P taken up by the skeleton will partly or wholly indicate an additional mineralization process and not replacement of old mineral constituents by new ones. It is therefore important that rats weighing more than 160 g. be used in experiments to determine rate of renewal. When such rats reach the old age period, they will lose some mineral constituents and some of the phosphorus given off may be labeled; this may lead to a low estimate of the extent of replacement which had taken place during the experiment. However, this source of error is far from being as dangerous as the first-mentioned type. When a rat grows from a weight of 110 g. to a weight of 140 g. the phosphorus content of the fresh femur increases from 5.3 to 7.3%, i.e., by about 38%. A substantial part of the femur can thus become labeled solely by additional mineralization, even if renewal of the mineral constituents is entirely lacking.

V. Adsorption Studies on Powdered Bone

Quantitative measurements of the adsorption of labeled phosphate ions by ground bone were carried out by Manly and Levy,[9] by Armstrong,[10] and by Hodge and associates.[11] The latter authors demonstrated that calcified tissues continued to adsorb ions for periods up to 64 hours with no indication of decreasing rates, and that diffusion was probably one of the controlling factors in the adsorption rates. Bone was found to adsorb more than dentine, which adsorbs more than

[9] M. Manly and S. Levy, *J. Am. Chem. Soc.*, **61**, 2588 (1939).

[10] W. D. Armstrong, *Proc. Soc. Exptl. Biol. Med.*, **44**, 28 (1940).

[11] H. C. Hodge, G. Van Hugsen, J. F. Bonner, and S. N. Van Voorhis, *J. Biol. Chem.*, **138**, 451 (1941). E. G. Johansson, M. Falkenheim, and H. C. Hodge, *ibid.*, **159**, 129 (1945). H. C. Hodge and M. Falkenheim, *ibid.*, **160**, 637 (1945).

enamel. Substantial amounts of phosphorus were found to have been taken up by the tissue. In a recent paper of Falkenheim *et al.,*[11a] the relative adsorption powers of enamel, dentine, apatite, and bone from a 2×10^{-3} *M* phosphate solution are stated to be 1, 5, 19, and 23, respectively, in a 10-day period. The decrease in the adsorbing power proceeds together with a decrease in the specific surfaces, which were found to be 99.3, 51.1, 2.4, and 1.8 m.[2] per gram. The adsorbing process is described as an exchange in which nearly one-fifth of the phosphorus atoms in the powdered solid bone may ultimately take part.

VI. Rate of Renewal of the Skeleton

A. EXPERIMENTS OF SHORT DURATION

If plasma or lymph containing labeled phosphate came into direct contact with all phosphate contained in the mineral constituents of bone tissue, a rapid interchange between bone phosphate and plasma or lymph phosphate would be expected to take place. The mineral constituents of bone, however, are incorporated in crystallites about 10^{-6} cm. in size,[12] which resemble those of the mineral apatite in constitution. It is the uppermost molecular layer of these crystallites which can come into direct contact with the fluid containing phosphate, a contact which will lead to interchange between the phosphate ions of the fluid and those of the uppermost molecular layer of the crystallites. Furthermore, new crystallites formed after the start of the experiment will contain labeled atoms. In the fully grown organism such a formation will go hand in hand with the dissolution of other crystallites or parts of crystallites, thus with a "biological" recrystallization.

As early as 5 minutes after the injection of 0.3 ml. physiological sodium chloride solution containing a negligible proportion of labeled sodium phosphate into the lymph sac of a 45-g. frog at 22° C., the tibia contained minute amounts of labeled phosphate.[13,14] Taking the specific activity of the plasma as 100, the specific activities of the epiphysis and diaphysis were 0.026 and 0.013, respectively. The ^{32}P content of the tibia increases with increasing time (Fig. 76).[13] After the lapse of

[11a] M. Falkenheim, W. F. Neuman, and H. C. Hodge, *J. Biol. Chem.,* **169,** 713 (1947).

[12] V. Caglioti, *Atti congr. naz., 5th Congr.,* **1,** 310 (1936).

[13] G. Hevesy, H. Levi, and O. H. Rebbe, *Biochem. J.,* **34,** 532 (1940).

[14] G. Hevesy, L. Hahn, and O. Rebbe, *Kgl. Danske Videnskab. Selskab Biol. Medd.,* **16,** 8 (1941).

1 hour, the specific activity of the epiphysis phosphorus amounts to only 1/600 of the corresponding value for plasma. Thus, less than 1/600 of the epiphyseal phosphorus is replaced by plasma phosphorus within 1 hour. During the following 3 hours, an additional part (1/900) of the epiphyseal phosphorus was exchanged. The first point in the curve was obtained by analyzing the right, the second point by analyzing the left, tibia. For the diaphysis, the corresponding figures were found to be 1/900 and 1/1200, respectively. Consequently, only a minute part of the tibia phosphorus was replaced by plasma phosphorus in the course of 4 hours. A still smaller replacement was found when the frog was kept at 0° C. The discrepancies found in the rates of replacement of the bone phosphorus of the rabbit (see page 417 and Neuman and Riley[14a]) and that of the frog are presumably due mainly to the lower temperature at which the experiments on frogs were carried out.

Fig. 76. Specific activity of tissue phosphorus at different times after injecting labeled phosphate in lymph sac of frog kept at 22° C.[14]

The low rate of replacement of bone phosphorus in the frog is illustrated by Figure 76, in which the rate of replacement of femur phosphorus is compared with the rate of replacement of gastrocnemius phosphorus. Despite the low rate of penetration of labeled phosphate into the gastrocnemius of the frog, the rate of replacement of tibial phosphorus after the lapse of 4 hours reached only about 1% of the corresponding value for gastrocnemius phosphorus.

It may require a long time for the circulating fluid to reach the surfaces of all or most of the apatite crystallites in the skeleton, and the rate at which the biological recrystallization takes place may be still appreciably lower. From Figure 76 it appears that shortly after administration of labeled phosphate, ^{32}P can already be detected in the skeleton.

When labeled phosphorus was injected drop by drop into the cir-

[14a] W. F. Neuman and R. F. Riley, *J. Biol. Chem.*, **168**, 545 (1947).

culation of a rabbit in a 3-hour experiment, the tibia diaphysis specific activity was only 0.27% of the plasma inorganic phosphorus activity at the end of the experiment.[14b] Corpuscle and muscle inorganic phosphorus were 11% and 6.9% of the plasma value, respectively.

In another set of experiments,[13] in which labeled phosphorus was injected intravenously into rabbits, the femur epiphysis showed 0.180% replacement of the bone phosphorus by labeled phosphorus in 2 hours and 0.200% replacement in 4 hours. The femur diaphysis had 0.056% replacement in 2 hours and 0.106% in 4 hours.

B. EXPERIMENTS OF LONG DURATION

The exchange between bone phosphate and plasma phosphate which has been observed in experiments of short duration, is presumably to be interpreted as due to replacement of the phosphate ions situated on the surface of the apatite crystals by those of the plasma or the lymph. If the surface exchange were exhausted, prolongation of the experiment would not be expected to affect the extent of replacement. If, however, in the course of time, formation of wholly or partly new apatite crystals took place, new and extensive possibilities of exchange between plasma phosphorus and bone phosphorus will arise. From these considerations it follows that incorporation of ^{32}P in bone apatite over a long period of time cannot be extrapolated from results obtained in experiments of short duration. The maximum ^{32}P uptake by the bones of old rats amounts to 5% of the dose administered.[14c] A greater percentage is found in young rats.[14a]

The usual way of applying radiophosphorus in the study of phosphorus turnover in the skeleton is the administration of labeled phosphate at the start of the experiment and the determination at the end of the experiment of the specific activity of the inorganic plasma phosphorus and the bone phosphorus. The specific activity of the plasma phosphorus declines very sharply in experiments of long duration. If we wish to calculate the extent of replacement, we must know the average values of the specific activity of the plasma phosphorus throughout the experiment. The fact that in the full-grown skeleton the *total* ^{32}P content does not increase greatly with time, except in the early phases of the experiment, indicates that some of the phosphorus must be firmly in the skeleton and some must be easily replaceable. As soon as the level

[14b] G. Hevesy and A. H. W. Aten, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **14**, 5 (1939).

[14c] L. H. Weissberger and P. L. Harris, *J. Biol. Chem.*, **151**, 543 (1943).

of plasma ^{32}P declines, radiophosphorus is drawn from the bone tissue (and other tissues) into the circulation, and a diminution of the *total* ^{32}P content of bone tissue results from this process. This is clearly shown in the results obtained by Manly and Bale[6] illustrated in Fig. 77 (*cf.*
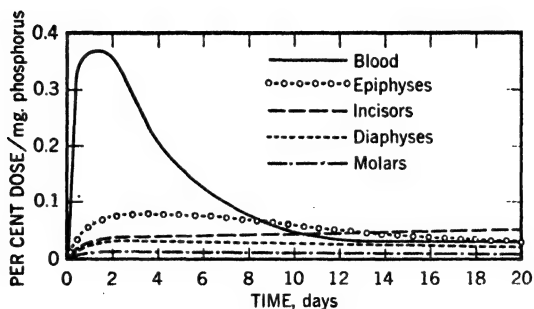
Fig. 77.   Change in ^{32}P content of bone tissue and blood of the rat with time.[6]

Manly *et al.*[15]). In these experiments changes in the specific activity of the phosphorus of the long bones and the corresponding values for blood were determined. It was found that with the decline of the activity level of the blood (and a still more pronounced decline can be expected in the activity of the plasma, not determined in these experiments) a decrease of the activity level of the epiphyses of the long bones took place. It is seen, furthermore, that in the incisors, which are typical representatives of rapidly growing tissue, ^{32}P is incorporated in a very different way. It is primarily incorporated with newly formed apatite crystals. The labeled phosphorus incorporated with these crystals does not respond, or responds at a very slow rate only, to changes in the activity level of the plasma. The rapid interchange process between plasma phosphate and easily interchangeable phosphate no longer dominates the interaction between this newly formed tissue and plasma phosphate.

The most direct approach to determining the extent of renewal of bone apatite was found to be the maintenance of the activity of the plasma phosphate at a constant level throughout the experiment and the comparison of the specific activity of the plasma phosphate at the end of the experiment with the specific activity of the bone mineral phosphate.[13] In order to maintain the plasma inorganic phosphorus at

[15] R. S. Manly, H. C. Hodge, and M. Manly, *J. Biol. Chem.*, **134**, 293 (1940).

a constant specific activity, labeled sodium phosphate of negligible weight was administered by subcutaneous injection to rabbits every 30 minutes on the first day, and later twice a day. After removal of the marrow, the bone was first extracted for 12 hours with hot ether–alcohol and subsequently treated with hot alkaline glycerol solution for 6 hours (the wet bones of a 3-kg. rabbit were found to comprise 8% of the total body weight[16]). The fractions obtained were dissolved in nitric acid and their phosphorus contents were precipitated as the ammonium magnesium salt. One aliquot of the sample obtained was used in the colorimetric phosphorus determination while another aliquot was reprecipitated as ammonium magnesium phosphate. In prolonged experiments, the analysis of plasma inorganic phosphorus was conveniently replaced by that of the urine phosphorus. In Tables 169 and 170, the specific activities of various bone phosphorus fractions are recorded.

·TABLE 169

Extent of Rejuvenation of Rabbit[a] Tibia in Nine Days[13]

Phosphorus fraction	P rejuvenated, %
Epiphysis	11.2
Diaphysis	3.2
Tibia phosphatide	74.8
Marrow phosphatide	80.1

[a] Rabbit weight = 2 kg.

In the course of 9 days, therefore, only 11% of the epiphysis and 3% of the diaphysis were rejuvenated, while most phosphatide molecules present in the marrow and in the bone were newly formed. Part of the phosphorus atoms present in the various fractions was presumably repeatedly renewed during the experiment. On this point, however, no information can be obtained from the above data. In 50 days, only 29% of the epiphysial and 7% of the diaphysial mineral constituents were replaced (Table 170). Tibia and femur showed about the same behavior. About half the scapula remained unchanged. The almost complete replacement of the apex and middle parts of the incisor dentine phosphorus can hardly be interpreted as due to simple exchange, since the replacement rate of the dentine phosphorus is normally even

[16] C. J. Levine, W. Mann, H. C. Hodge, I. Ariel, and O. Du Pont, *Proc. Soc. Exptl. Biol. Med.*, **47**, 318 (1941).

lower than that of the tibia phosphorus. The high ^{32}P content of the incisor dentine must be ascribed to actual growth, with new bone formation by an active calcification process. As a plasma containing ^{32}P was instrumental in calcifying the newly grown parts of the incisor, the phosphorus of the latter was bound to have the same specific activity as was shown by the plasma phosphorus. As seen from Table 170, the phosphorus of the apex part of the incisor dentine investigated had, within the errors of experiment ($\pm 5\%$), the same specific activity as

TABLE 170

Extent of Rejuvenation of Rabbit Skeleton in Fifty Days[13]

Phosphorus fraction	Rejuvenation, %
Femur epiphysis inorganic..............	29.7
Femur epiphysis phosphatide...........	100
Femur diaphysis inorganic..............	6.7
Femur diaphysis phosphatide........... About 100
Tibia epiphysis inorganic..............	28.6
Tibia diaphysis inorganic..............	7.6
Ribs................................	27.5
Scapula.............................	43.8
Incisor dentine, apex.................	103
Incisor dentine, middle...............	98.5
Incisor dentine, tip..................	41.2
Incisor enamel, apex + middle..........	82.0
Incisor enamel, tip...................	6.6

had the plasma phosphorus. This part (about 0.9 mm. long), was entirely newly formed during the experiment. The bulk of the middle part of the dentine was also freshly grown, while the incisal tip, having a length of 1.2 mm., was only partially newly formed with participation of the labeled plasma. About half the phosphorus atoms present in the incisal part of the dentine have not been replaced; they must thus have been located in the apex or middle regions of the incisor before the start of the experiment. The tissue containing these atoms was pushed forward *in toto*. We witness here a case of tissue formation in which macroscopic aggregates are moved *in toto* as the incisor tooth grows, undergoing only restricted atomic or molecular replacement. This effect is more clearly shown in the growth of the enamel.

The apex and middle parts of the enamel, being formed by a cal-

cification process from labeled plasma, became strongly active. From the fact that the incisal part of the enamel is only slightly active, we may conclude that this fraction was not formed through a calcification process in the course of the experiment. Its crystals must have been formed earlier from nonlabeled plasma, and the whole fraction must have moved *in toto* during the experiment from the position in which it had been calcified into the place in which it was found at the end of the experiment. Also the incisal end of the dentine may probably be formed to a large extent from the nonlabeled tissue. This conclusion, however, is not supported as clearly as by the activity figures found in the case of enamel. Part of the preformed incisal dentine tissue appears to have become labeled to an appreciable extent during the growth process. Preformed enamel tissue becomes labeled only to a minute extent (see page 426).

VII. Biological Recrystallization

Biological recrystallization is made possible by the fact that the concentration of phosphate, calcium, and other constituents which play an essential part in the calcification process varies in the plasma and the lymph. Intake of food increases the phosphate and the organic phosphorus compound contents of the plasma and the lymph, and so do numerous biochemical processes which cause enzymic splitting of organic phosphorus compounds. Plasma phosphate, for example, increases after intense muscular action, although the low phosphate permeability of the muscle cells much reduces phosphate releases. Estrogens increase calcium and phosphorus in the blood of oviparous vertebrates during the reproductive cycle. Excretion of phosphorus, on the other hand, acts in the opposite direction, as do numerous other biochemical processes in the course of which phosphate is incorporated with organic compounds; among these processes the decrease in the phosphate content of the plasma by the action of insulin possibly is the most conspicuous one.

It is not only the phosphate and the equally important calcium concentration of the plasma that fluctuates, but also the concentration of phosphatase and other enzymes which regulate phosphorus metabolism. Repeated administration of bone phosphatase extracts by intravenous injection was found[17] to cause a decrease in the mineral constituents of bone tissue. Phosphatases act on bone formation both by regulating

[17] H. C. Hodge, E. Gavett, and I. Thomas, *J. Biol. Chem.*, **163**, 1 (1946).

the phosphate concentration of the plasma and probably also in a more direct way, as was suggested by Robison[18] in his early studies on bone formation, discussed on page 409, and by the work of Roche.[19] More recent work by Roche and Morgue[20] indicates that a fracture of the rat femur involved dissolution of appreciable amounts of the mineral constituents of the femur followed by the opposite process after about one month. During the first weeks, phosphatase activity of the bone was also enhanced. Roche and Morgue, moreover, have made the observation that fracture of the left femur not only led to an initial decrease in the mineral constituents of the fractured left bone, later followed by a reversal of this process, but that the right femur also exhibited similar behavior. They suggest that the enhanced phosphatase activity of bone tissue may possibly be attributed to enhanced magnesium concentration produced by osteolysis, following the fracture. Thus, even fluctuation in the magnesium content of the plasma would promote the rate of renewal of the skeleton.

Concerning the calcium content of the skeleton, King[21] emphasizes that, conventionally, the bony framework of the body is regarded as a means for making locomotion possible, but that this might be no more than a secondary development, the primary function of the bone in the body being to act as reservoir for the maintenance of a constant blood calcium. Bauer and associates[22] have furthermore shown that the number of bone trabeculae increases and decreases under various conditions, so that calcium and phosphorus can be stored or released, as the need arises.

VIII. Mobilization of the Mineral Constituents of Hen Skeleton

Some of the mineral constituents of the skeleton are mobilized during shell formation in the laying bird. The ratio of calcium (1 gram) to phosphorus (3–4 milligrams) in the shell is much higher (about 300 times) than in the skeleton; obviously, therefore, the extra phosphorus must be deposited elsewhere. In the frequently laying bird, the rapidly growing ova have access to this extra phosphorus, which is responsible for an increase in the plasma phosphorus from 4.2 to 7.5 milligram

[18] R. Robison, *The Significance of Phosphorus Esters in Metabolism*, 1912.

[19] J. Roche and G. Deltour, *Compt. rend.*, **216**, 748 (1943).

[20] J. Roche and M. Morgue, *Bull. soc. chim. biol.*, **21**, 143 (1939).

[21] J. B. King, *Brit. J. Radiology*, **12**, 2 (1939).

[22] W. Bauer, J. C. Aub, and F. Albright, *J. Exptl. Med.*, **49**, 145 (1929).

per cent. In birds which lay infrequently much of the extra phosphorus is excreted. This fact explains the finding[23] that phosphorus excretion in the droppings of hens increased during egg shell formation in birds on a diet containing 0.67% calcium or less, and it is of interest to note that Lorenz and co-workers[24] found that hens laying infrequently showed periods of increased ^{32}P excretion that coincided with periods of shell formation. Obviously, when the hen's diet contains 2.2% calcium, bone calcium is no longer mobilized to supply the calcium necessary for shell formation. There would appear to be a mechanism whereby the laying hen is prevented from losing too much calcium from the skeleton on laying days. The laying hen can prevent the daily withdrawal of more than 1 g. calcium from her bone.

IX. Migration of Phosphate from Soft Tissues to the Skeleton

As is seen in Table 171, the labeled phosphate, which in early phases of the experiment is largely found in the soft tissues, with time is present almost exclusively in the skeleton. This observation supplies additional evidence that biological recrystallization of the skeleton takes place at a slow rate. As the activity level of the plasma decreases with time, labeled phosphate in the later phases of the experiment moves from the tissues, in which it is present at a higher concentration than in the plasma, into the circulation.

TABLE 171

Percentage of Total Body ^{32}P Found in Muscles and Skeleton of Rats[25]

| | Time after ^{32}P administration | | | | | | |
| | Hours | | Days | | | | |
Organ	0.5	4	10	20	30	50	98
Muscles..........	18.3	19.4	25.8	28.8	25.2	12.1	3.6
Total skeleton.....	19.1	13.4	43.1	43.1	51.8	76.5	92.0

The labeled phosphate in the uppermost molecular layers of bone apatite crystals will promptly follow the changes in the activity level of the plasma. Not so, however, the labeled phosphate incorporated with the more deeply situated molecular layers of the skeleton, because

[23] R. H. Common, *J. Agr. Sci.*, **22**, 576 (1932); **23**, 555 (1933); **26**, 86 (1936).

[24] F. W. Lorenz, I. Perlman, and I. L. Chaikoff, *Am. J. Physiol.*, **138**, 318 (1942/43).

[25] G. Hevesy, *J. Chem. Soc.*, **1939**, 1213.

TABLE 173

Chemical Composition of Adult Human Body[27]

Parts analyzed	Per cent of total body	Chemical composition						
		Water, %	Ether extract, %	Crude protein (N × 6.25), %	Ash, %	Calcium, %	Phosphorus, %	Heat of combustion, cal./g.
Skin	7.81	64.68	13.00	22.19	0.68	0.0205	0.060	2.292
Skeleton	14.84	31.81	17.18	18.93	28.91	11.02	4.83	2.497
Teeth	0.06	5.00[a]		23[a]	70.90	24.42	11.81	
Striated muscle	31.56	79.52	3.35	16.50	0.93	0.0099	0.116	1.239
Brain, spinal cord, and nerve trunks	2.52	73.33	12.68	12.06	1.37	0.0188	0.352	1.905
Liver	3.41	71.46	10.35	16.19	0.88	0.0102	0.148	2.196
Heart[b]	0.69	73.69	9.26	15.88	0.80	0.0078	0.113	1.824
Lungs[c]	4.15	83.74	1.54	13.38	0.95	0.0116	0.114	0.985
Spleen	0.19	78.69	1.19	17.81	1.13	0.0079	0.217	1.193
Kidneys	0.51	79.47	4.01	14.69	0.96	0.0130	0.174	1.326
Pancreas	0.16	73.08	13.08	12.69	0.93	0.0143	0.155	1.979
Alimentary tract	2.07	79.07	6.24	13.19	0.86	0.0125	0.115	1.339
Adipose tissue	13.63	50.09	42.44	7.06	0.51	0.0116	0.048	4.165
Remaining tissues								
Liquid	3.79	93.33	0.17	5.68	0.94	0.0054	0.066	0.382
Solid	13.63	70.40	12.39	16.06	1.01	0.0675	0.053	2.040
Contents of alimentary tract	0.80							
Bile	0.15							
Hair	0.03							
Total body, weighing 70.55 kg.	100.00	67.85	12.51	14.39	4.84	1.596	0.771	1.930

[a] Assumed.   [b] Somewhat enlarged.   [c] Somewhat congested.

the rate of biological recrystallization is slow, and even in experiments lasting some months a large part of the bone apatite remains unchanged.

X. Uptake of ^{32}P by the Human Skeleton

No data on the rate of renewal of human bone apatite are available. However, Erf[26] determined the uptake of ^{32}P by various parts of the skeleton and by other organs of a 65-year-old human subject who received 20 millicuries [32]P 19 days before death. The subject died of leukemia. The ^{32}P uptake by different parts of the skeleton (non-mineral constituents not being removed) is seen in Table 172 (*cf.* page 116).

TABLE 172

Uptake of ^{32}P by Different Parts of a Human Skeleton Nineteen Days after Administration of 20 Millicuries[26]

Bone	Microcuries per gram wet weight
Calverium	0.01
Femur diaphysis	0.01
Femur epiphysis	0.02
Ribs	0.16
Tibia diaphysis	0.02
Sternum	0.12
Vertebral bodies	0.08

Assuming the skeleton to weigh 12 kg. (see Table 173), about 5% of the ^{32}P administered can be estimated to be present in the skeleton. The chemical composition of the adult human body is seen in Table 173.[27]

Radioactive phosphorus has been used in the study of phosphorus balance in patients with resistant rickets.[27a] Further data on the uptake of ^{32}P by the human skeleton are stated on pages 115 and 116.

XI. Rate of Renewal of Fish Skeleton

To investigate the rate of renewal of the fish skeleton,[28] *Gasterosteus aculeatus* (weighing 1 to 2 g.) was kept in sea water containing labeled

[26] L. A. Erf, *Proc. Soc. Exptl. Biol. Med.*, **47**, 287 (1941).

[27] H. H. Mitchell, T. S. Hamilton, F. R. Steggerda, and H. W. Bean, *J. Biol. Chem.*, **158**, 625 (1945).

[27a] L. Gunther, E. T. Cohn, W. E. Cohn, and D. M. Greenberg, *Am. J. Diseases Children*, **66**, 517 (1943).

[28] G. Hevesy, *Acta Physiol. Scand.*, **9**, 234 (1945).

phosphate (0.25 mg. phosphorus per liter). The labeled sea water was renewed daily. At the end of the experiment, the specific activity of the mineral phosphorus of the skeleton was compared with the specific activity of the inorganic liver phosphorus. This procedure was used because of the difficulty of securing blood samples from small fish. Exchange equilibrium between the inorganic phosphorus of the liver and the inorganic phosphorus of the plasma is reached after a short time and, thus, measurement of the latter value can be replaced by determining the specific activity of the inorganic phosphorus of the liver. The extent of renewal of the mineral constituents of the skeleton of the fish in one month was found to be less than 5%. The low rate of renewal of fish skeleton is presumably due to the low temperature (16° C.) prevailing during the experiment. A low rate of renewal of frog skeleton was also observed at 22° C., and a still lower one at 0° C.[14]

XII. Uptake of ^{32}P by Dentine and Enamel

The rate of replacement of the phosphorus in rat molars, which contain both dentine and enamel, is appreciably lower than in the long bones, the maximum uptake by 1 g. long bone ash[29] amounting to 5 times the corresponding figure for 1 g. molar ash.[32] As expected, the loss in ^{32}P from the molars in later phases of the experiments concerning this replacement is correspondingly slower than the loss from the long bones.

The percentages of the total quantity of radiophosphorus administered by subcutaneous injection to cats by Barnum and Armstrong[33] (see also Armstrong[34]) in hard tissues and marrow are shown in Table 174.

As was to be expected, the dentine behaved as did the diaphysial bone tissue. A very minute percentage of the administered ^{32}P is taken up only by the enamel.

[29] The ash of the bones of the rat makes up 39% of the fresh weight, while the corresponding figure for the teeth of the rat is 61%.[30] The ash of rabbit bone is 32.6% of the fresh weight.[31]

[30] E. Watchorn and R. A. McCance, *Biochem. J.*, **31**, 1379 (1937).

[31] M. J. L. Dols, B. C. P. Jansen, G. J. Sizoo, and F. Barendregt, *Koninkl. Nederland. Akad. Wetenschap. Proc.*, **41**, 1 (1938).

[32] M. Manly and W. F. Bale, *J. Biol. Chem.*, **129**, 125 (1939).

[33] C. P. Barnum and W. D. Armstrong, *Am. J. Physiol.*, **135**, 478 (1942).

[34] W. D. Armstrong, *Ann. Rev. Biochem.*, **11**, 441 (1942).

The ^{32}P content of enamel is conditioned partly externally, by contact with the oral secretion, and partly internally, via pulp and dentine, as found by different workers[33][36] (see Table 175, taken from a paper by Sognnaes and Volker[36]).

TABLE 174

Percentage Radiophosphorus (Administered by Subcutaneous Injection to Cats) Found after Five Days[33]

Tissue	Per cent of administered ^{32}P per gram ash
Enamel	0.000546
Dentine	0.00878
Femur diaphysis	0.0133
Femur epiphysis	0.0611
Femur marrow	0.311

In other experiments, the enamel of dog molars protected by caps was found to take up 10 to 12% of the amount of ^{32}P retained by

TABLE 175

Uptake of Radiophosphorus by Dog Teeth as Influenced by Saliva[36]

Type of sample	^{32}P, per cent of total dose per gram	
	Left jaws (covered)	Right jaws (stimulated saliva)
Surface enamel	0.37×10^{4}	23.0×10^{-4}
Remaining enamel	1.65	2.95
Dentine–enamel junction	6.67	2.19
Crown dentine	41.5	42.8
Root dentine	54.9	55.2
Submaxillary gland	15.65	46.50

the enamel of uncapped molars.[37] The uptake of ^{32}P from the saliva becomes about 100 times larger when part of the enamel is missing.[38]

[34a] J. F. Volker and R. F. Sognnaes, *J. Dental Research*, **19**, 242 (1940).

[35] P. O. Pedersen and B. Schmidt-Nielsen, *Schweiz. monatsh. Zahnheilkunde*, **51**, 647 (1941).

[36] R. F. Sognnaes and J. F. Volker, *Am. J. Physiol.*, **133**, 112 (1941).

[37] H. Berggren, *Acta Radiol.*, **27**, 248 (1946).

[38] J. R. Blayney, F. Wasserman, G. Groetzinger, and T. G. DeWitt, *J. Dental Research*, **20**, 559 (1941).

One week after administration of labeled sodium phosphate to a human subject, about 1/300,000 of the ^{32}P administered was found in each tooth.[5] In these experiments the pulp was not removed. Since food phosphate is absorbed at a similar rate as sodium phosphate added to the food, we can expect about 1/300,000 of the food phosphate to be present in each tooth after 7 days.

In their investigation of the dentine of the crown and the root of a tooth extracted from a 14-year-old girl, after removal of the pulp and predentine, Pedersen and Schmidt-Nielsen[35,39] found 1/500,000 and 1/800,000, respectively, of the ^{32}P administered orally after the lapse of 2 to 23 days.

Barnum and Armstrong[33] have shown that labeled phosphate migrates outwardly from enamel and dentine in experiments of long duration. They found that, 116 days after subcutaneous injection of labeled sodium phosphate, the activity in the enamel of the distal half of rat incisors was higher than in the dentine.

XIII. Effect of Vitamin D on Deposition of Phosphorus in Rachitic Metaphysis

Vitamin D was found by Morgareidge and Manly[40] (see also Bale[41]) to have no influence on the entrance of orally administered labeled phosphorus into the blood or into the tibia diaphysis in normal animals. In rachitic metaphyses, however, the effect of vitamin D can be clearly demonstrated (see Fig. 78). The effects in the metaphyses are first seen after the lapse of 54 to 72 hours following the administration of vitamin D. The entrance of phosphorus into this structure corresponded almost exactly to the appearance of the "line" produced by silver nitrate staining in animals which had received a single dose of vitamin D about 54 or more hours previously. In the metaphyses of the control animals, the "line" did not appear and the content of radioactive phosphorus did not increase. This evidence supports the conclusion that vitamin D has a direct influence on calcification only in rachitic animals (see page 434). Cohn and Greenberg[42] also used radioactive phosphorus to study the effects of vitamin D on rachitic rats.

[39] P. O. Pedersen and B. Schmidt-Nielsen, *Acta Odontol. Scand.*, **4**, 1 (1942).
[40] K. Morgareidge and M. Manly, *J. Nutrition*, **18**, 411 (1939).
[41] W. F. Bale, *Radiology*, **35**, 184 (1940).
[42] W. E. Cohn and D. M. Greenberg, *J. Biol. Chem.*, **130**, 625 (1939).

Shimotori and Morgan[43] concluded from experiments in which a single large dose of vitamin D was administered along with doses of ^{32}P that vitamin D exerts its effect more by intensification of the phos-
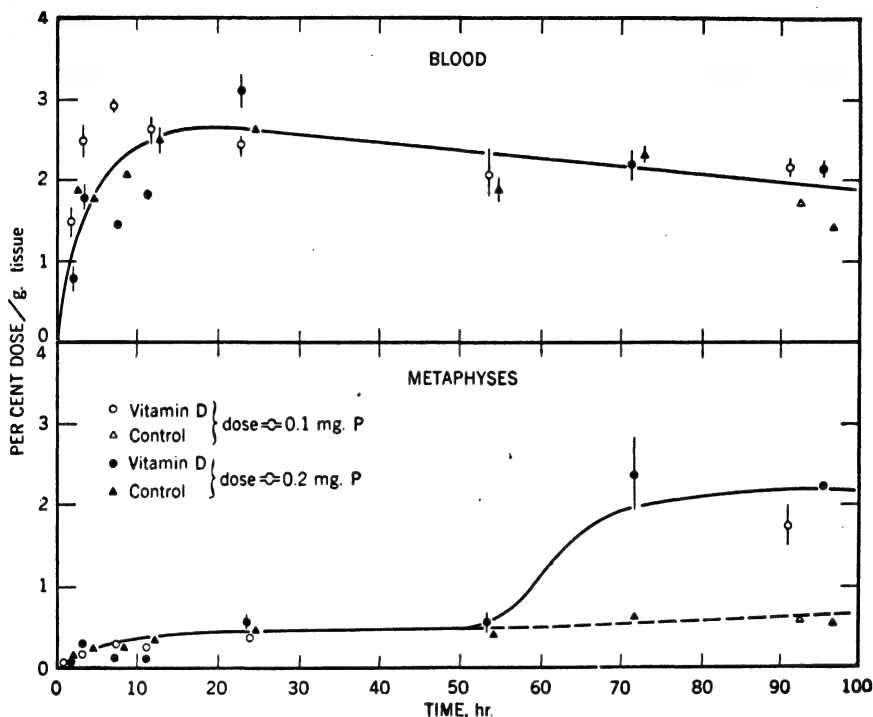
Fig. 78.   Entrance of ^{32}P into blood and metaphyses of the rat.[40]

phorus turnover in the bone, accompanied by hyperphosphatemia and decreased visceral phosphorus turnover, than through increased absorption of phosphorus from the gut.   The ^{32}P uptake by the femur of the dog was found by them to be increased almost twofold after administration of a massive dose of vitamin D.   In these experiments vitamin D was fed in the form of irradiated ergosterol and delsterol at the level of 200,000 I.U. per kg. of body weight.   The oils were fed by mouth and, immediately following the dosing, labeled sodium phosphate was administered by stomach tube.   The ^{32}P content of numerous organs was determined 72 hours later.   The large dose of vitamin D caused decreased uptake of ^{32}P in almost all the soft tissues, as seen in Table 175A.

Administration of a massive dose of vitamin D had no significant

[43] N. Shimotori and A. F. Morgan, *J. Biol. Chem.*, **147**, 201 (1943).
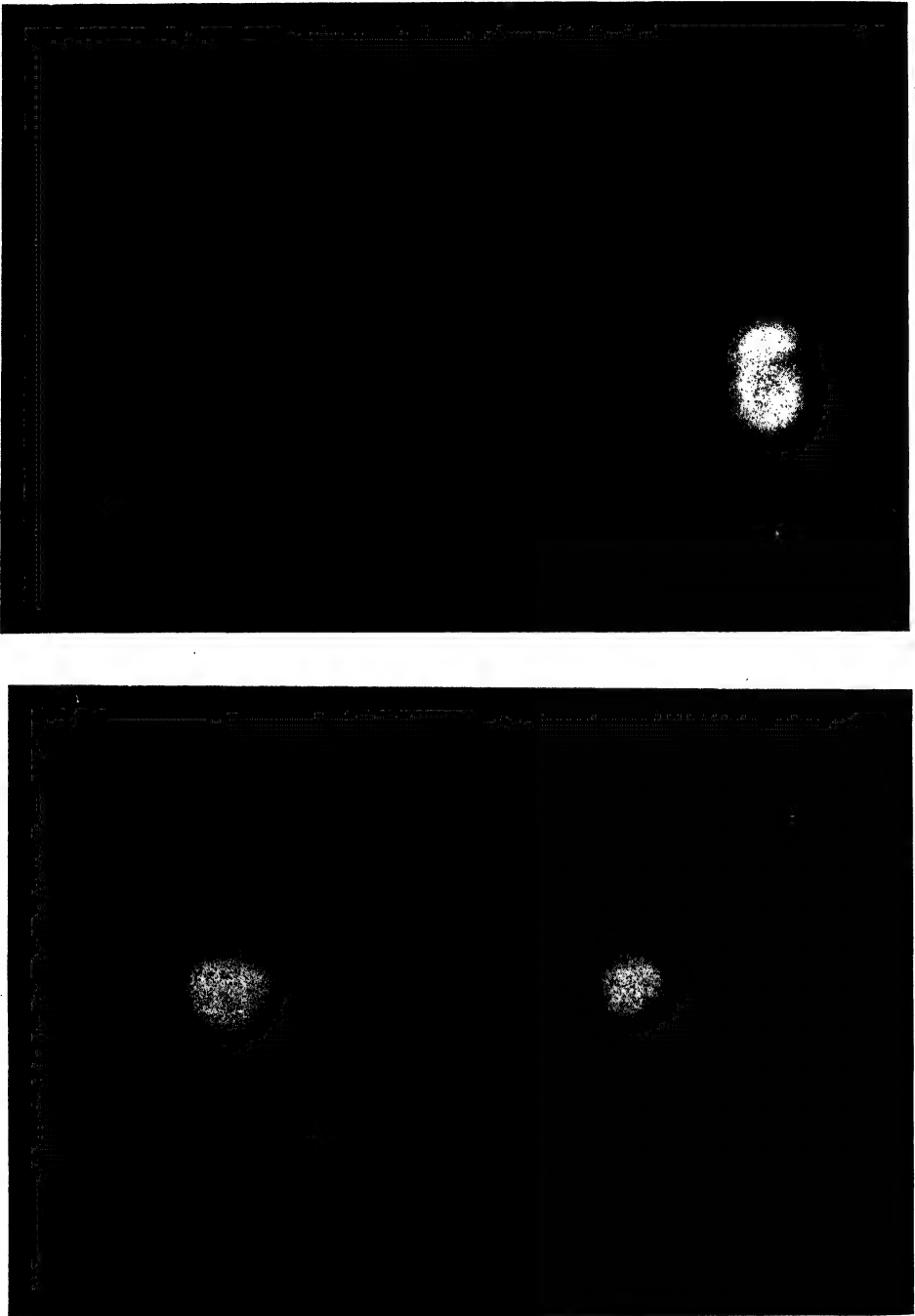
Fig. 79. Radioautograph of the tibia of a normal (above)
and of a rachitic (below) chicken.[44]

effect on the rate of disappearance of labeled phosphate from the blood stream. There was a marked increase in the urinary phosphate excretion.

TABLE 175A

Distribution of ^{32}P in Tissue of Dogs without Vitamin D
and with a Large Dose of Vitamin D[43]

Tissue	^{32}P content per g. dog tissue, per cent of dose		Specific activity $\times 10^{-2}$	
	Without vitamin D	With vitamin D	Without vitamin D	With vitamin D
Spleen	0.067	0.039	6.2	4.9
Stomach	0.050	0.030	5.2	4.0
Small intestine	0.089	0.075	7.2	5.7
Large intestine	0.054	0.048	7.0	5.7
Liver	0.088	0.057	9.0	6.1
Kidney	0.071	0.063	6.5	5.0
Adrenals	0.069	0.052	6.3	5.2
Ovaries	0.054	0.036	5.1	3.8
Heart	0.051	0.037	6.0	3.8
Lungs	0.059	0.057	5.8	4.5
Brain	0.008	0.008	0.5	0.5
Muscle	0.034	0.024	5.5	3.1
Fat	0.004	0.001	6.0	4.1
Skin and hair	0.018	0.005	4.7	2.0
Femur ash	0.036	0.069	0.2	0.4

When ^{32}P was fed in the diet, Dols et al.[31,44,44a] observed a higher uptake of ^{32}P by bones of rachitic chicks as compared with normal, as determined by direct measurement and by radioautographs as seen in Figure 79.

Excessive vitamin E intake, 100 mg. of natural mixed tocopherols per rat per day, was found to cause an increase in ^{32}P uptake by the bone of 4- to 19-week-old animals, while a moderate vitamin E intake, 10 mg. of tocopherol per day, brought about no change in phosphorus metabolism.[45]

Between the 8- and 18-hour interval after intraperitoneal administration of radioactive phosphorus, the femurs of rats treated with

[44] M. J. L. Dols, B. C. P. Jansen, G. J. Sizoo, and G. J. van der Maas, Koninkl. Nederland. Akad. Wetenschap. Proc., **42**, No. 6, 1 (1939).

[44a] M. J. L. Dols, B. C. P. Jansen, G. J. Sizoo, and G. J. van der Maas, Koninkl. Nederland. Akad. Wetenschap. Proc., **40**, 547 (1937).

[45] L. H. Weissberger and P. L. Harris, J. Biol. Chem., **151**, 543 (1943).

parathyroid extract, began to lose the ^{32}P content more rapidly than those of the untreated controls. This accelerated movement of ^{32}P from the femurs of the treated rats results presumably from the sustained action of parathyroid extract.[45]

XIV. Effect of Röntgen Rays on ^{32}P Uptake by Bone

Marinelli[46] irradiated one leg of 3-month-old albino mice with 3000 r. of 190 kv. unfiltered Röntgen rays. The rest of the animal was covered with 3-mm. lead sheet. Some of the mice were retained as
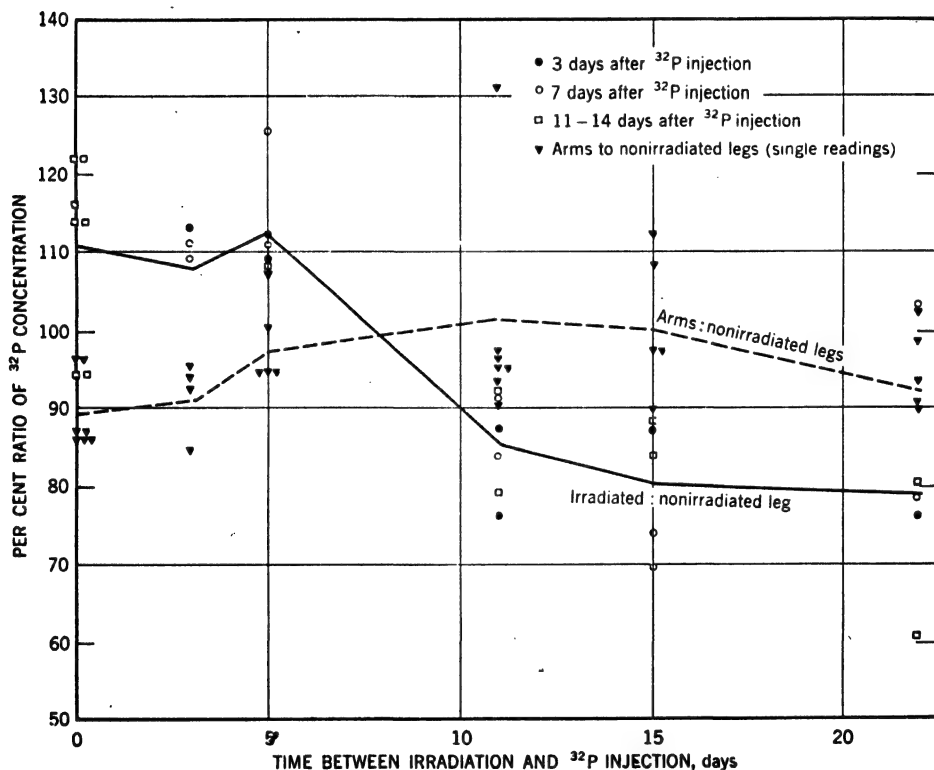
Fig. 80.   Effect of Röntgen rays on retention of ^{32}P by legs of mice.[47]

controls. After the irradiation which took only 3 minutes all animals were given tracer doses of ^{32}P interperitoneally and were sacrificed at different times thereafter. If the ^{32}P was injected into the animal

[46] L. D. Marinelli, *personal communication*. See also L. D. Marinelli and J. M. Kenney, *Radiology*, **37**, 691 (1941).

[47] C. L. Hinkel, *Am. J. Roentgenol. Radium Therapy*, **47**, 439 (1942).

several days after irradiation of one of its legs, the irradiated leg was found to contain only about 70% of the radiophosphorus content of the nonirradiated leg, as seen in Figure 80. This difference was not observed when the administration of ^{32}P took place immediately after irradiation. These results are consistent with the view that the ^{32}P interchange between bone and plasma is impaired and that this damage does not immediately follow x-ray irradiation, but becomes more pronounced after a latent period of 1 to 3 weeks.[47]   In these experiments no difference in ^{32}P uptake per gram weight was detected in irradiated muscle as compared to nonirradiated muscle. The average ratio of the first to the second, as ascertained in 21 determinations involving 84 animals, was 0.95 and the standard error of the mean was 0.05. Nor was a significant difference found in irradiated skin as compared to non-irradiated.

XV. Rate of Replacement of Bone Calcium

While we can expect to obtain similar results for the rate of biological recrystallization of bone apatite with labeled calcium as indicator and with radiophosphorus as the tracer, the rate of renewal of Ca ions of the uppermost molecular layer of the crystals may differ from the corresponding value found for the phosphate ions.   C. Pecher[48] (cf. Pecher[48a]) has compared the percentage uptake of ^{45}Ca and ^{32}P in the bone and other tissues of fully grown mice. He found that 24 hours after intravenous administration of ^{45}Ca and ^{32}P, respectively, the ratio of uptake by bone and muscle tissues of equal fresh weights was 66.7 and 2.3, respectively.   That a much larger percentage of the ^{45}Ca than of the ^{32}P administered was found in the bone tissue is explained by the comparatively low calcium content of the soft organs, and not by a correspondingly higher rate of calcium turnover in the skeleton. Part of the ^{45}Ca and ^{89}Sr previously fixed in the skeleton of mice was found to migrate to the fetus during the last days of pregnancy.

Campbell and Greenberg[49] have found that, 69 hours after administration of labeled calcium lactate to fully grown rats, 1 g. of dry bone tissue takes up 130 times as much ^{45}Ca as is taken up by 1 g. of dry muscle tissue (see page 155).

The mode of action of vitamin D in the healing of rickets was investi-

[48] C. Pecher, *Proc. Soc. Exptl. Biol. Med.*, **46**, 86 (1941).

[48a] C. Pecher and J. Pecher, *Proc. Soc. Exptl. Biol. Med.*, **46**, 91 (1941).

[49] W. W. Campbell and D. M. Greenberg, *Proc. Natl. Acad. Sci. U. S.*, **26**, 176 (1940).

gated by Greenberg[50] by means of tracer experiments with calcium and strontium. Vitamin D was found to exert its healing effect partly by favoring the absorption of calcium from the intestinal tract, and partly by a direct influence on the process of mineralization in bone. The partition of labeled calcium and strontium in the body and excreta of rachitic and vitamin D-treated rats is seen in Tables 176 and 177.

TABLE 176

Partition of Labeled Calcium and Strontium in Body and Excreta
of Rachitic and Vitamin D-Treated Rats[50]

| Mode of administration | Per cent of total dose[a] | | | | Residual carcass |
	Urine	Feces	Skeleton	Teeth	
	Strontium				
Oral, no vit......	21.3 ± 2.1	67.0 ± 1.9	9.5 ± 2.1	1.3 ± 0.5	1.2 ± 0.45
Oral, with vit....	32.6 ± 3.3	48.8 ± 1.8	15.0 ± 3.7	1.35 ± 0.1	2.1 ± 0.7
Injected, no vit...	61.8 ± 2.6	21.0 ± 1.9	11.1 ± 0.4	2.6 ± 0.8	2.3 ± 0.4
Injected, with vit.	48.8 ± 2.1	17.3 ± 4.4	26.0 ± 6.8	2.5 ± 0.25	4.0 ± 1.4
	Calcium				
Oral, no vit......	19.5	60.0	15.0	2.1	3.4
Oral, with vit. ...	29.4	32.5	31.0	3.5	3.6
Injected, no vit...	44.0	18.5	28.0	6.5	3.0
Injected, with vit.	25.0	18.0	45.0	6.0	6.0

[a] Values are means ± the mean deviations of four animals in each group sacrificed 3 days after abeled strontium administration. The values for animals given radiocalcium are means for 2 rats.

Doses of 0.2 to 0.3 ml. of irradiated ergosterol (10,000 U.S.P. XI units of vitamin D per gram) or cottonseed oil were administered orally to rachitic rats 72 hours and 1 hour prior to administration of the radioactive materials. The data of Table 176 show that one of the actions of vitamin D is to promote the absorption of calcium from the intestinal tract. The injected animals in a state of healing accumulate nearly twice as much ^{45}Ca or ^{89}Sr per gram of dry bone and about 1.5 times as much per gram of bone ash. The influence of vitamin D on the retention of calcium by the teeth is small in comparison with its influence on bone. A similar result may apply to all the diaphysial parts of the skeleton.

[50] D. M. Greenberg, *J. Biol. Chem.*, **157**, 99 (1945).

Weissberger and Harris[51] found that vitamin D increased greatly the retention of strontium. The retention of radiostrontium by bones was also found to be increased by injecting phosphate into the rat.[52] One injection of 500 Hanson units of parathyroid extract produces no effect upon the retention of radiostrontium. The injection of 500 units 24 hours before injecting 500 additional units with labeled strontium results in a decreased retention and increased urinary excretion of radiostrontium. In these experiments, 2 mg. labeled strontium, having an activity of 3 to 15 microcuries, was administered. Data on the uptake of radiostrontium by the bones are also given by Posin.[53]

Strontium is absorbed from strontium chloride solutions by various calcified tissues in the following order: bone > dentine > enamel.[17] The adsorption of strontium by powdered bone *in vitro* was found to be quite appreciable.

XVI. Uptake of Radium and Lead

The successive slow increase in radium and lead content of bone tissue with time, when these elements are present in the circulation even in traces, also indicates a very slow rate of biological recrystallization of bone apatite crystals. Just as ^{32}P administered and not yet excreted finds its way to the skeleton to an increasing extent with increasing time, radium present in the organism, after the lapse of some time, is found almost exclusively in the bone. A 52-year-old man had drunk water containing radium for about 5 years. Autopsy revealed a total radium content of 73.7 μg. The soft tissues contained only 0.53% of the total radium content, while the rest was found in the skeleton, the uptake per gram of vertebra ash being especially pronounced, as seen in Table 178.[54] Since the affinity of radium for phosphate is greater than that of calcium, radium has a chance of being preferentially incorporated into the newly formed apatite crystallites. The possibility that some radium might be precipitated as very insoluble radium sulfate, and be present as such, should however not be discounted. In a similar way, increase in the lead content of human bones with age can be explained.[55]

[51] L. H. Weissberger and P. L. Harris, *J. Biol. Chem.*, **144**, 287 (1942).

[52] W. R. Tweedy, *J. Biol. Chem.*, **161**, 105 (1945).

[53] D. Q. Posin, *Proc. Montana Acad. Sci.*, **3**, 10 (1942); **4**, 15 (1943).

[54] A. O. Gettler and C. Norris, *J. Am. Med. Assoc.*, **100**, 400 (1933).

[55] H. P. Morris, *J. Ind. Hyg. Toxicol.*, **22**, 100 (1940).

TABLE 177

Accumulation of Radioactive Strontium and Calcium in Bone and Teeth of Rachitic and Vitamin D-Treated Animals[50]

Mode of administration	Radiostrontium to 6 rats			Radiocalcium to 2 rats		
	Femur ash, g.	Per cent dose per gram		Femur ash, g.	Per cent dose per gram	
		dry femur	femur ash		dry femur	femur ash
Oral, no vit.	31.4 ± 5.2	3.9 ± 1.0	12.5 ± 4.0	30.3	6.6	22
Oral, with vit.	34.1 ± 8.0	5.6 ± 1.5	16.5 ± 4.3	32.4	13.6	42
Injected, no vit.	30 ± 4.7	5.4 ± 1.0	18 ± 2.6	30.1	12.6	42
Injected, with vit.	37 ± 8.0	9.8 ± 1.7	26.5 ± 6.8	35.8	25.1	71

Mode of administration	Radiostrontium to 3 rats			Radiocalcium to 2 rats		
	Incisor dry wt. g.	Uptake by whole incisor	Uptake/g. dry incisor	Molar dry wt. g.	Uptake by whole molar	Uptake/g. dry molar
Oral, no vit.	0.15	0.92	6.2	0.13	0.20	1.6
Oral, with vit.	0.15	0.98	6.6	0.12	0.26	2.2
Injected, no vit.	0.17	1.34	8.0	0.14	0.30	2.2
Injected, with vit.	0.19	1.60	8.6	0.11	0.34	3.3

Mode of administration	Radiocalcium to 2 rats		
	Incisor dry wt. g.	Uptake by whole incisor	Uptake/g. dry incisor
Oral, no vit.	0.14	2.1	15.1
Oral, with vit.	0.17	3.5	20.6
Injected, no vit.	0.17	5.5	32.3
Injected, with vit.	0.15	4.2	28

Administered by subcutaneous injection, radium was found to accumulate in the skeleton of the rat in the first 96 hours, but after that time some radium was released (see page 159). The accumulation of radium in the skeleton of the mouse or the rat after administration of 0.008 mg and 0.05 mg., respectively, is also shown by radioautographs.[56]

TABLE 178

Distribution of Radium in Human Bones and Tissues[54]

Tissues	Ra, µg./g. ash	Tissues	Ra., µg./g. ash
Femur............	0.0092	Heart............	0.0008
Vertebrae........	0.0342	Spleen...........	0.0009
Rib.............	0.0039	Kidneys..........	0.0046
Jaw bone........	0.0076	Liver............	0.0012
Teeth...........	0.0149	Lungs...........	0.0008

When the lead isotope thorium B was injected, 26% was found in the skeleton after 6 hours, and 67% was found after the lapse of 68 hours.[57]

XVII. Uptake of Sodium, Potassium, and Zinc

The sodium content of bone is greatly in excess of the amount accounted for by the extracellular fluid of bone.[58] The excess sodium is found to be proportional to the calcium content, 1 molecule of sodium being found for 30 molecules of calcium. The excess sodium of bones cannot be extracted by prolonged digestion with alcoholic potassium hydroxide solution and subsequent extraction with water. The suggestion is, therefore, offered that the sodium found in calcified material is part of an apatite complex similar to sodium found in naturally occurring fluoroapatite.[60]

In experiments by Berggren[59] in which 20 millicuries of radiosodium were injected into the dog, the teeth were protected from contact with saliva by a rubber dam. In 100 mg. enamel about $1/10^8$ of the activity administered was found to be present. The uptake of ^{24}Na by the enamel of molars is clearly shown in the radioautographs in Figure 81.

[56] F. Daels, H. Fajerman, and Van de Putte *Strahlentherapie*, **63**, 545 (1938).

[57] C. Dittmar, *Z. Krebsforsch.*, **48**, 121 (1939).

[58] H. E. Harrison, *J. Biol. Chem.*, **120**, 457 (1937).

[59] H. Berggren, *Acta Radiol.*, **27**, 248 (1946).

[60] H. E. Harrison, D. C. Darrow, and H. Yannet, *J. Biol. Chem.*, **113**, 515 (1936).

These were obtained of ground sections with uniform thickness of 200–300 μ. The time of exposure was 1 week; Eastman spectroscopic plates were used. Practically identical activity figures were obtained when measuring the activity of the upper enamel layer or of the deep enamel layer obtained by grinding from within the enamel of molars influenced by the saliva.
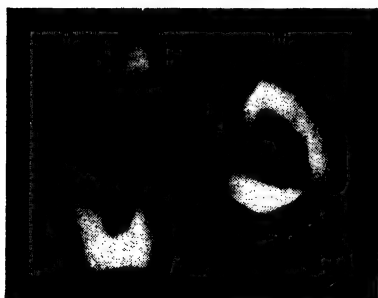
Fig. 81. Uptake of radiosodium by the enamel of dog molars (*courtesy* Dr. H. Berggren).[59]

Results obtained by Hodge and associates in experiments *in vitro* with radiosodium[61] indicate the operation of an adsorption isotherm in the uptake of sodium by the bone. These authors regard bone sodium as a constituent adsorbed on the surface of the apatite crystals. The incorporation of sodium in the apatite crystal can, however, not be excluded.[62]

The specific activity of the bone potassium of the rabbit 24 hours after administration of labeled potassium amounts to only 25% of the specific activity of the plasma potassium.[63] This finding suggests that a substantial part of the bone potassium is located in the apatite crystals.

The value for radiozinc found in the bones of mice 160 hours after intravenous injection is twice the value found after 2 hours indicating slow incorporation of zinc into the bone tissue.[64]

XVIII. Fluoride Content and Rate of Renewal of the Skeleton

In view of the great similarity between hydroxyl ions and fluoride ions, the hydroxyl ions of the apatite structure can be replaced by fluoride ions. The fluoride content of bone apatite crystals is to a large extent determined by the fluoride content of the plasma at the time of their formation. Since the hydroxyl ions present in the uppermost molecular layer of the crystallites can be expected to interchange promptly with plasma fluoride, the fluoride content of this molecular

[61] H. C. Hodge, W. F. Koss, J. T. Ginn, M. Falkenheim, E. Gavett, R. C. Fowler, I. Thomas, J. F. Bonner, and G. Dessauer, *J. Biol. Chem.*, **148**, 321 (1943).

[62] L. Hahn, G. Hevesy, and O. Rebbe, *Biochem. J*, **33**, 1549 (1939).

[63] W. O. Fenn, T. R. Noonan, L. J. Mullins, and L. Haege, *Am. J. Physiol.*, **135**, 149 (1941/42).

[64] G. E. Sheline, I. L. Chaikoff, H. B. Jones, and M. L. Montgomery, *J. Biol. Chem.*, **149**, 139 (1943).

layer will be determined by the fluorine concentration of the plasma prevailing at the end of the experiment.

The fluoride content of the plasma is mainly determined by the fluoride content of the food. This is shown by the experiments of Klement,[65] who compared the fluoride content of the skeleton of animals living on land with the skeleton of animals living in the sea. The fluoride content of the earth's crust is much lower than that of the sea water and, correspondingly, the fluoride content of the food of animals living on land is much lower than that of animals living in the sea. The fluoride content of the plasma of salt water fishes is also increased by the fluoride present in the sea water which these fishes drink. Table 179 gives a summary of the results obtained by Klement.

TABLE 179

Percentage Fluoride Content of the Mineral Constituents
of the Skeleton[65]

Animal	Land	Sea	Fresh water
Mammals.........	0.05	0.55	
Birds............	0.11	0.32	
Fishes...........		0.43	0.03

The skeleton of fish living in the Baltic, which has a low salt and low fluoride content, contains much less fluoride (0.06%) than the skeleton of fish living in the Atlantic. By moving fish, such as *Pleuronectes flesus*, from the Baltic into the Atlantic and measuring the time necessary for the fish skeleton to reach the fluoride content (or, for example, half the fluoride content), of *P. flesus* normally living in the Atlantic, we can calculate the rate of renewal of the skeleton in a manner similar to the calculation for the rate of incorporation of radiophosphorus.

When growing rats were kept on a diet containing sodium fluoride, after the lapse of 32 weeks, a fluoride content of 0.94% was found in the fat-free bones. After the addition of fluoride to the food had ceased, in one group of the rats investigated the fluoride content was reduced from 0.943 to 0.302% in 12 weeks; in another group it decreased from 0.60 to 0.287% in 14 weeks. Thus, in 13 weeks two-thirds to one-half the fluoride present in the bones was found to be replaced. These figures represent the upper limit of the extent of renewal of the bone

[65] R. Klement, *Ber.* **68**, 2012 (1935).

apatite in the course of 12–14 weeks. Increase in the fluorine content of the diet to about 40 times its normal value for a year leads to an increase in the fluorine content of human dentine from 0.026 to 0.045%.[66]

In the ash of the bones of cryolite workers employed continuously in cryolite factories, up to 1.3% fluorine was found to be present. Thus, about one-third the hydroxyl ions were replaced by fluoride ions.[67] This conclusion is based upon the assumption that all fluorine present in the skeleton replaced apatite hydroxide. The possibility of the formation of complex phosphofluorides should, however, not be excluded.

Radiofluoride, having a half-life of only 112 minutes, can only be used in experiments of short duration. In 35 minutes after intraperitoneal injection of ^{18}F to mice, 1 g. of femur shaft was found to contain three times as much ^{18}F as 1 g. of blood, indicating a rapid uptake of ^{18}F by the skeleton. In the determination of the uptake of ^{18}F by the bones of the cat much lower figures were found.[68]

Powdered enamel, dentine, and bone were exposed for 30 minutes at 40° C. to solutions of sodium fluoride varying in concentration from $1:100$ to $1:10^{6}$. Radioactive fluoride was added to each solution. The logarithm of the amount of fluoride picked up per gram of the various calcium phosphates was plotted against the logarithm of the concentration of the fluoride remaining in the solutions. In each case, a straight line was obtained, satisfying this criterion of adsorption.[69]

XIX. Deposition of Radiocarbon in Bone

Solomon and co-workers[70] found that part, at least, of the bone carbonate is readily formed from plasma bicarbonate (or possibly replaced by other carbonate ions). They found also that, after the lapse of 2.5 hours, 1.8% of the labeled bicarbonate (0.3 to 0.5 mg.) administered to rats was present in the bone carbonate.

The radioautographs of the bones of rats injected with ^{14}C show a markedly different picture[71] from those of bones obtained after injection

[66] J. F. McClendon and W. C. Foster, *Federation Proc.*, **2**, 33 (1943).

[67] K. Roholm, *Fluorine Intoxication.* H. K. Lewis, London, 1937.

[68] J. F. Volker, R. F. Sognnaes, and B. G. Bibby, *Am. J. Physiol.*, **132**, 707 (1941).

[69] J. F. Volker, H. C. Hodge, H. Wilson, and S. N. Van Voorhis, *J. Biol. Chem.*, **134**, 543 (1940).

[70] A. K. Solomon, B. Vennesland, F. W. Klemperer, J. M. Buchanan, and A. B. Hastings, *J. Biol. Chem.*, **140**, 171 (1941).

[71] W. Bloom, H. J. Curtis, and F. C. McLean, *Science*, **105**, 45 (1947).

of ^{32}P or ^{89}Sr. The ^{14}C injected as carbonate appears primarily in those areas occupied by preexisting bone. It does not appear in appreciable quantities in the areas of most recently deposited bone salt. The radioautographs of the 2-, 4-, 8-, and 16-week specimens show essentially the same picture as those after 3 days, except that the bones have grown in length and width. While it would appear that there is approximately as much ^{14}C in the bones after 16 weeks as after 3 days, there was a great decrease in the ^{14}C content of the soft tissue. To what extent the ^{14}C is present as a constituent of organic and inorganic constituents, respectively, is yet to be elucidated.

XX. Uptake of Fission Products and Plutonium by the Skeleton

Many of the radioactive elements formed by fission of uranium are found to localize in the skeleton. Because of their radioactivity, they constitute a potential health hazard quite similar to that from radium poisoning.

The metabolism of the radioisotopes of strontium, yttrium, and cerium formed by fission, and of the transuranic element plutonium, has been investigated by Copp, Axelrod, and Hamilton.[72] Of these elements, only strontium was absorbed to any appreciable extent from the intestinal tract. Absorption of radioactive strontium from the intestines was 25 times as great in growing rats on a low calcium diet as in adult rats receiving ample calcium in the diet.

In contrast, neither activity of new bone formation nor state of calcium depletion appeared to have any effect on the behavior of yttrium, cerium, and plutonium. Severe phosphorus deficiency, which caused a threefold reduction in the retention of radioactive strontium (associated with the inhibition of bone formation) also had no apparent effect on the distribution of yttrium, cerium, and plutonium (see Table 180). These results suggest that only the alkaline earth strontium follows the path of calcium metabolism, while yttrium, cerium, and plutonium are deposited by some other mechanism.

This view was given further support by the study of Van Middlesworth, Copp, and Hamilton on the uptake of yttrium, strontium, and plutonium by healing bone fractures.[73] Peak uptake of yttrium and

[72] D. H. Copp, D. J. Axelrod, and J. G. Hamilton, *Am. J. Roentgenol. Radium Therapy*, **58**, 10 (1947).

[73] L. Van Middlesworth, D. H. Copp, and J. G. Hamilton, *Federation Proc.*, *in press*.

Fig. 82. Femur from rat weaned to phosphorus-deficient diet at three weeks, injected intraperitoneally with 5 microcuries of strontium at five weeks, and sacrificed one week later. Note that the strontium is deposited almost exclusively in the bone salt of the shaft, with practically none in the uncalcified osteoid matrix below the epiphysis.[72]

plutonium took place on the second day before any mineralization of the callus had taken place; uptake of strontium, insignificant before the fourth day, reached a peak at 8–12 days when calcification was most active.

Radioautographs of undecalcified bone sections were prepared by the technique of Axelrod[74] from animals injected with strontium, yttrium, cerium, and plutonium. Of particular interest were the radio-autographs of bones from rats with severe phosphorus deficiency. Because of the extreme demineralization of the skeleton, there were

Fig. 82A. Femur from rat weaned to phosphorus-deficient diet at three weeks, injected intramuscularly with 20 micrograms plutonium at five weeks, and sacrificed at six weeks. Note heavy deposits of plutonium in the uncalcified osteoid matrix below the epiphysis, and superficial deposition of plutonium in the shaft.[72]

[74] D. J. Axelrod, *Anat. Record, in press.*

large areas of uncalcified organic matrix below the epiphysis which were free of bone salt. Radioactive strontium (Figure 82) was found only in regions where bone salt was still present; yttrium, cerium, and plutonium (Figure 82A) were also laid down in the areas of uncalcified organic matrix.

<div align="center">TABLE 180</div>

<div align="center">Effect of Phosphorus Deficiency on Retention of ^{89}Sr, ^{239}Pu, ^{91}Y, and ^{144}Ce by the Rat[72]</div>

Element[a]	Diet	Per cent of dose absorbed	Per cent of absorbed dose in		
			Carcass	Urine	Feces
Sr	Control	100	76.3	5.6	8.3
	P deficient	100	25.8	36.5	12.7
Pu	Control	30.3	80.1	2.8	6.0
	P deficient	58.0	83.9	3.7	6.8
Y	Control	57.9	75.7	12.7	5.4
	P deficient	64.5	74.1	8.4	11.5
Ce	Control	85.0	60.5	5.7	12.0
	P deficient	89.0	71.9	4.8	5.0

[a] ^{89}Sr injected intraperitoneally; ^{239}Pu, ^{91}Y, and ^{144}Ce injected intramuscularly.

The usual procedures advocated for chronic radium poisoning, such as low calcium diet, ammonium chloride, parathormone, and citrate, had no significant effect on the chronic elimination of these elements. However, a method for reducing the toxicity of the plutonium in the body has been suggested by Copp *et al.*[72]

Rats injected with plutonium were placed for 6 weeks on a diet designed to promote resorption. They were then fed for 6 weeks a diet high in calcium, phosphorus, and vitamin D to promote new bone formation. Radioautographs of the femur showed little or no radioactivity in the new bone, which now covered the plutonium deposits and shielded the bone marrow from the short range α-particles. Data on uptake of plutonium by bone are also found on page 167.

CHAPTER XI

Application of Radioactive Indicators in the Study of Red Corpuscles

I. General Remarks

Isotopic indicators have found extended application in the study of the permeability and the metabolic processes of the red corpuscles and in the labeling of these cells. Labeled corpuscles are used in determining the amount of circulating corpuscles, their life cycle, and in investigating numerous other problems. We shall first consider the application of radiophosphorus.

II. Turnover of Phosphorus in Red Corpuscles

A. EXPERIMENTS OF SHORT DURATION

The ratio of the inorganic phosphorus content of the red corpuscles and that of the plasma can be expected to be about equal to the corresponding value for chloride, thus, equal to 0.52.[1] Brain and his associates [2] have found that human corpuscles and plasma contain 4.1 and 2.4 milligram per cent inorganic phosphorus, respectively. Snyder et al.[2a] state that for the inorganic P content of human plasma values varying between 5.4 and 7 milligram per cent, the corresponding corpuscle values vary between traces and 5.9 milligram per cent. In the corpuscles of the rat, 6.6 and 3.8 milligram per cent, respectively, are values given.[3] Varying values were found by different workers. This is due partly to variations in the phosphate concentration of the corpuscles, and partly to analytical difficulties. Additional formation of organic phosphorus compounds in the corpuscles will diminish the inorganic phosphorus content, possibly within too little time to replace the phosphorus incorporated in organic molecules by plasma phosphorus, and vice versa. Shift in pH of the blood from normal toward greater

[1] E. G. Weir and A. B. Hastings, *J. Biol. Chem.*, **129**, 547 (1939).

[2] R. T. Brain, H. D. Kay, and P. G. Marshall, *Biochem. J.*, **22**, 628 (1928).

[2a] R. Snyder and S. Katzenelbogen, *J. Biol. Chem.*, **143**, 223 (1942).

[3] S. Rapoport and G. M. Guest, *J. Biol. Chem.*, **126**, 749 (1938).

acidity leads, for example, to a rapid decrease in concentration of diphosphoglycerate and also, to a minor extent, of other acid-soluble phosphorus compounds present in the corpuscles,[4] while alkalosis created, for example, by overbreathing, leads to some increase in the concentration of organic phosphates in the corpuscles. As to analytical difficulties, during the extraction process organic phosphorus compounds may split off inorganic phosphate, or vice versa.

The acid-soluble organic phosphorus content of the plasma varies within wide limits. In normal human plasma, values varying between 0.0 and 0.9 milligram per cent with an average value of 0.33 milligram per cent were recorded. Much larger amounts were found in the corpuscles, the human corpuscles on an average containing 46 milligram per cent, rat 67 milligram per cent, and rabbit corpuscles as much as 88 milligram per cent.[5-7] A smaller difference is found between the phosphatide phosphorus content of the corpuscles and the phosphatide phosphorus content of the plasma, corpuscles containing about twice as much phosphatide as plasma of equal weight. Human corpuscles and plasma contain 19 and 9 milligrams per cent, respectively. In contradistinction to mammalian corpuscles, avian corpuscles also contain appreciable amounts of nucleic acid phosphorus.[5]

As to the nature of the phosphoric esters present in the corpuscles, various compounds have been reported, such as adenosine triphosphate, hexose phosphate, triose phosphate, mono- and diphosphoglycerate, glycerophosphate, and phosphopyruvate; avian corpuscles contain appreciable amounts of phytate phosphorus as well.[8] In human blood corpuscles about 51% of the ester phosphorus is present as phosphoglycerate, 21% as hexose phosphate, and 24% as adenosine triphosphate.[5] In rabbit corpuscles, of 88 milligrams per cent of total organic acid-soluble phosphorus present, 51% is diphosphoglycerate phosphorus and 24% is adenosine triphosphate phosphorus, a large part of the remaining fraction being hexose monophosphate phosphorus.[9]

After administering labeled phosphate or shaking a blood sample in the presence of labeled phosphate, radiophosphorus can be found after

[4] S. Rapoport and G. M. Guest, *J. Biol. Chem.*, **138**, 269 (1941).

[5] S. Rapoport and G. M. Guest, *J. Biol. Chem.*, **129**, 781 (1939).

[6] G. M. Guest and S. Rapoport, *Physiol. Revs.*, **21**, 410 (1941).

[7] S. E. Kerr, *J. Biol. Chem.*, **117**, 227 (1937).

[8] S. Rapoport, *J. Biol. Chem.*, **135**, 403 (1940).

[9] E. Warweg and G. Stearns, *J. Biol. Chem.*, **115**, 567 (1936).

a short interval both in the inorganic phosphorus and in the ester phosphorus of the corpuscles. The specific activity of corpuscular pyrophosphate phosphorus, after the lapse of a few minutes, is almost as high as that of the inorganic phosphorus extracted from the corpuscles; both, however, have specific activities much lower than the specific activity of inorganic phosphorus extracted from the plasma. This fact indicates that the rate of passage of phosphate into the corpuscles is low, while the phosphate ions which penetrate the corpuscles rapidly participate in phosphorylation processes.[10-12a] During glycolytic processes going on in the corpuscles, "old" ester molecules are split off and new ones are formed. Since radiophosphorus is present in the corpuscles, it participates in the formation of "new" ester molecules.

Early studies already yielded evidence that the maintenance of a normal level of adenosine triphosphate and other phosphoric esters in the blood is due to their continued synthesis and decomposition during glycolysis. Dische[13] later showed that glucose was phosphorylated in blood only through the agency of adenosine triphosphate and that fructose diphosphate and triose phosphate were intermediary products formed during glycolysis. This author emphasized the apparent self-regulatory character of the reactions comprising the glycolytic cycle in the blood. Guest and Rapoport [14] have presented a scheme to demonstrate the principal steps of the glycolytic cycle in blood, as suggested by the theories of Embden, Meyerhof, Parnas, Dische, and others. They emphasize that the glycolytic process has an important biological function apart from the breakdown of sugar: namely, the regulation of the concentration at which these phosphorus compounds are carried into the blood under different conditions.

By making use of radiophosphorus as an indicator we can follow the formation in the corpuscles of new organic phosphorus molecules of

[10] G. Hevesy and A. H. W. Aten, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **14**, No. 5 (1939).

[11] L. Hahn and G. Hevesy, *Acta Physiol. Scand.*, **3**, 193 (1942).

[12] A. J. Eisenman, L. Ott, P. K. Smith, and A. W. Winkler, *J. Biol. Chem.*, **135**, 165 (1940).

[12a] L. Hahn and G. Hevesy, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, **22**, 188 (1938).

[13] Z. Dische, *Biochem. Z.*, **274**, 51 (1934); 280, 248 (1935); *Enzymologia*, **1**, 288 (1936).

[14] G. M. Guest and S. Rapoport, *Am. J. Diseases Children*, **58**, 1072 (1939).

various types and determine the speed of their formation. Some of these types of molecules as, for example, adenosine triphosphate, are renewed at such a high speed that only the lower limit of the rate of formation can be stated.

Figure 83 shows that within 90 minutes half the organic acid-soluble phosphorus molecules of the corpuscles are renewed, *i.e.*, their phosphate content is replaced during an enzymic process. In this figure, the results of experiments are shown in which rabbit blood was shaken in the presence of negligible amounts of labeled sodium phosphate,[10] while the results of other similar experiments are given in Table 181. The figures show that in the course of 30 minutes a very large part of the phosphorus split off by 7-minute hydrolysis (see Warweg and Stearns[9]), composed of pyrophosphate, is renewed. This fraction contains both the first and the second labile phosphorus atoms of the adenosine triphos-

Fig. 83. Extent of renewal of total acid-soluble phosphorus in red corpuscles of the rabbit.[10]

TABLE 181

Experiments *in Vitro* with Rabbit Blood[15]

Corpuscle fraction	Duration of experiment, min.	Specific activity at end of experiment
Inorganic P...................	30	100
Hydrolyzed 7 min..............	30	77
Hydrolyzed 7 min. to 12 hr......	30	16
Nonhydrolyzed................	30	13
Inorganic P...................	60	100
Hydrolyzed 7 min..............	60	90
Hydrolyzed 7 min. to 12 hr......	60	41
Nonhydrolyzed................	60	28

phate molecule. Recent work performed on the relative rate of renewal of the muscular adenosine triphosphate phosphorus (see page

[15] G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **15**, No. 7 (1940).

257) showed that the second labile phosphorus atom of these molecules is split off at a slower rate than the first labile phosphorus atom, the ratio of renewal of the three phosphorus atoms of the adenosine triphosphate molecule of rat muscle being 100:75:5. Similar considerations probably apply to the adenosine triphosphate phosphorus present in the red corpuscles; and 75% may be considered a lower limit of the extent of renewal of the terminal phosphorus of the adenosine triphosphate molecule. A large part of these phosphorus atoms is renewed several times in the course of 30 minutes (see Table 181).

The rate of renewal of phosphoric esters was found to be reduced with increasing acidity of the blood. Changes in pH toward greater alkalinity tend to produce effects opposite to the effects of increased acidity. The changes in pH may be assumed to involve mainly the phosphorylation of glucose by adenosine triphosphate.[6]

Rabbit blood contains about 4 mg. pyrophosphate phosphorus per 100 g. Since, in the course of 30 minutes, 75% or more of this pyrophosphate is renewed, the lower limit of the amount of new adenosine triphosphate molecules formed during this time is 3 milligram per cent, or 0.1 millimole per cent. As the oxygen consumption of the blood of the rabbit in the course of 30 minutes amounts to less than 0.01 millimole per cent,[16] the formation of new adenosine triphosphate molecules can hardly be directly connected with the oxygen consumption.

We can, however, expect the new formation of adenosine triphosphate molecules to be involved in glycolytic processes, each phosphorylation first going through adenosine triphosphate and thus necessitating a renewal of the labile phosphorus of the adenosine triphosphate molecules. Colowick[17] has found that at least 10 atoms of phosphate are esterified per oxidized glucose molecule, and as stated above during the consumption of 1 molecule of oxygen at least 10 molecules of pyrophosphate are turned over.

B. EXPERIMENTS OF LONG DURATION

When comparing the specific activity of plasma inorganic phosphorus with that of corpuscle inorganic phosphorus in experiments in which the activity of the inorganic phosphate of the plasma is kept at a constant level throughout the experiment, after 11.5 hours (see Table 182), this ratio is found to be 4 for rabbit blood, showing that the rate

[16] L. Michaelis and K. Salomon, *Naturwissenschaften*, **18**, 566 (1930).

[17] S. P. Colowick, *J. Biol. Chem.*, **137**, 343 (1941).

of penetration of phosphate ions from the plasma into the corpuscles, and vice versa, is slow, a much slower process than the rate of renewal of most acid-soluble organic phosphorus compounds present in the corpuscles. After the lapse of 9 days, the ratio of the specific activity of inorganic phosphorus and corpuscle average acid-soluble phosphorus

TABLE 182

Rate of Incorporation of ^{32}P in the Phosphorus Compounds
of the Red Corpuscles[15]

Corpuscle fraction	Specific activity[a]
11.5 hours	
Inorganic P	25
Hydrolyzed P, 1 N H_2SO_4, 100°C., 120 hr.	25
Acid-soluble residual P	13
9 days	
Total acid-soluble P	94
50 days	
Total acid-soluble P	100

[a] Plasma inorganic phosphorus taken as 100. By repeated administration of labeled phosphate, ^{32}P content of the inorganic phosphate of the plasma was kept at constant level.

is only slightly greater than 1 (1.06) — after such a long time, the activity of the average corpuscle acid-soluble phosphorus reached almost the same value as the inorganic phosphorus in the corpuscles — and, after the lapse of 50 days, a completely proportional distribution of the labeled phosphorus atoms between plasma phosphorus and acid-soluble phosphorus in the corpuscles is attained.[15]

The interesting phenomenon — that an individual phosphate ion, although penetrating the corpuscles fairly slowly, is incorporated at a remarkable rate into organic molecules in the corpuscles — finds many analogies in the processes going on in various organs. It is strikingly shown in the study of the penetration of labeled phosphate into muscle cells and in experiments on rate of renewal of the acid-soluble phosphorus compounds of these cells; the former process is slow; the latter process, in the case of some compounds, is very rapid (see page 238).

The specific activity of the blood hexose monophosphate of the guinea pig was not influenced by insulin, epinephrine, or glucose. The same negative result was obtained in the investigation of inorganic phosphorus and of the barium-soluble and the alcohol-insoluble phosphorus fraction of the blood.[18]

C. NUCLEATED CORPUSCLES

Guest and Rapoport[6] have also demonstrated that an unidentified phosphoric ester, comprising about 5–10% of the organic acid-soluble phosphorus in human and rabbit blood, also participates in glycolysis. Preliminary experiments with radioactive phosphorus indicate that this fraction is rapidly renewed, and other findings suggest that it may be a precursor of phosphoglyceric acid.

In mammalian corpuscles, adenosine triphosphate is continuously resynthesized during glycolysis. In nucleated red corpuscles, on the other hand, Engelhardt[19] has·found that adenosine triphosphate is preserved only if respiration can take place.

In man and other animals whose red corpuscles contain large amounts of diphosphoglycerate, this substance has been found to be important in several respects. This was especially stressed by Guest and Rapoport,[20] who state that it is formed and decomposed as an intermediate product of blood glycolysis. As a nondiffusible anion in the corpuscles it is an important factor in the maintenance of ionic equilibrium in the blood, and it appears to serve various functions in phosphorus metabolism. The rate of renewal of the diphosphoglycerate present in the corpuscles (see Table 184) is lower than the rate of renewal of the easily hydrolyzable fractions, but is still quite pronounced.

In contradistinction to mammalian corpuscles, the avian and turtle corpuscles contain no diphosphoglycerate but phytic acid is present in large amounts, i.e., from 40–75% of the organic acid-soluble phosphorus present.[4] Phytic acid appears not to participate in the glycolytic process, but to undergo a slow aerobic turnover. The extent of renewal of phytic acid phosphorus in goose corpuscles incubated at 37.5° C. for 24 hours was found, by Rapoport et al.,[21] to be about 2.5%. This result was obtained after shaking goose blood to which labeled Na_2HPO_4

[18] L. H. Weissberger, J. Biol. Chem., **160**, 481 (1945).

[19] W. A. Engelhardt, Biochem. Z., **227**, 16 (1930).

[20] G. M. Guest and S. Rapoport, Am. J. Diseases Children, **58**, 1072 (1939).

[21] S. Rapoport, E. Leva, and G. M. Guest, J. Biol. Chem., **139**, 633 (1941).

was added at 37.5° C. in an oxygen atmosphere. When the blood was incubated in a nitrogen atmosphere, or when anaerobic conditions were obtained by adding potassium cyanide in sufficient amount to give a concentration of approximately 0.003 N potassium cyanide, or when the blood was saturated with carbon monoxide, much lower values were

TABLE 183

Rate of Renewal of Phosphorus Compounds of Goose Blood[21]

Conditions	Incubation, time, hr., at 37.5° C.	Radioactivity per mg. P compared with that of acid-soluble P other than phytate P, %		
		Phytate P	Phosphatide P	Residual P
O_2	24	2.50		
O_2	24	2.17		
O_2	24	2.72	1.79	0.89
N_2	24	0.019	0.008	0.01
KCN	24	0.03		
KCN	24	0.18		
KCN	24	0.03	0.08	0.08
CO	24	0.001	0.000	0.01

TABLE 184

Specific Activity of Phosphorus Fractions of Rabbit Blood Hemolyzate at 37° C. 2 Hours after Labeled Phosphate Addition[11]

Phosphorus fraction	P content, mg.	^{32}P distribution, %	Specific activity
Inorganic	4.45	85	100
Hydrolyzed, 0 to 7 min.	2.06	9.2	23.5
7 to 100 min.	3.79		5.8
7 min. to 17 hr.	10.88	5.37	2.58
Residual acid-soluble	1.62	0.33	1.07
Total organic acid-soluble	14.6	14.9	6.29

found for the rate of renewal of phytate phosphate (see Table 183). Anaerobic conditions had a similar effect on the rate of renewal of phosphatides.

D. FORMATION OF LABELED ACID-SOLUBLE PHOSPHORUS COMPOUNDS IN BLOOD HEMOLYZATES

When labeled phosphate is added to blood hemolyzates, some formation of labeled acid-soluble phosphorus compounds takes place,

but the extent of renewal is much smaller than in the intact corpuscles (see Table 185). Hemolyzates were obtained by repeatedly cooling the blood corpuscles to liquid air temperature.

In the intact corpuscles, after the lapse of 1 hour, the activity of the phosphorus obtained by hydrolysis with 1 N acid for 12 hours after the removal of the pyrophosphate amounted to 41% of the activity of the inorganic phosphorus of the corpuscles. In the hemolyzate, in the course of 2 hours, the corresponding figure was only 5.8%.

That the rate of resynthesis of organic phosphorus is markedly reduced in the hemolyzate follows also from the fact that the hemolyzate contains more inorganic phosphorus and less organic phosphorus than intact blood. As shown by using labeled phosphorus as an indicator, alternating degradation and resynthesis of adenosine triphosphate and also of other organic phosphorus compounds takes place in the corpuscles and also in the hemolyzate. In the hemolyzate the resynthesis obviously lags behind the degradation, and the inorganic phosphorus content accordingly increases in the course of the experiment. The rate of resynthesis of organic phosphorus compounds in the hemolyzate is considerably reduced by lowering the temperature. As seen in Table 185, at 0° about 25% as many labeled organic phosphorus compounds were synthesized as at 37°.

TABLE 185

Effect of Temperature on Formation of Labeled Organic Phosphorus Compounds in Rabbit Blood Hemolyzate[11]

Time, hr.	Temperature, °C.	Per cent ^{32}P found in organic fraction	P content, milligram per cent		Specific activity	
			Inorganic	Organic	Inorganic	Organic
1	37	11.6	9.38	15.0	100	8.20
1	0	3.34	9.05	15.3	100	2.00
2	37	17.2	5.55	18.9	100	5.09
2	0	7.10	3.76	20.6	100	1.20

Lowering the rate of formation of labeled adenosine triphosphate in the hemolyzate may be due partly or wholly to the destruction of cozymase in the hemolyzate. In a hemolyzate of the corpuscles of horse blood, Lennerstrand[22] found, 3 hours after hemolysis, 40%

[22] Å. Lennerstrand, *Arkiv Kemi Mineral. Geol.*, **A14**, No. 16 (1941).

destruction of the cozymase present in the corpuscles. Lennerstrand found also that the increase in inorganic phosphate content of the hemolyzate is due at least partly to dephosphorylation of adenosine triphosphate (see also Rapoport and Guest[23]).

E. EFFECT OF POISONS ON ENZYMES RESPONSIBLE FOR SYNTHESIS OF ORGANIC PHOSPHORUS COMPOUNDS IN CORPUSCLES

In normal corpuscles, the penetration of ^{32}P into the corpuscle is followed by incorporation into organic molecules which, in turn, give off nonlabeled phosphorus. By this process, the corpuscle inorganic phosphorus is for quite a while prevented from attaining a high specific activity. If, however, formation of organic phosphorus compounds is hindered, and this would be the case in poisoned corpuscles, the ^{32}P migrating into the corpuscle would remain in the inorganic phosphorus fraction; this fraction would soon become strongly active. Rabbit blood was shaken with different volumes (see Table 186) of an isotonic potassium cyanide solution ($pH = 7.5$ to 8.0) at 37° C. for some time previous to the addition of labeled phosphate of negligible weight. As seen in the table, the distribution of ^{32}P among the various phosphorus compounds in the corpuscles was different in the controls and in the cyanide-treated erythrocytes. In the corpuscles treated with cyanide, most of the ^{32}P present was found in the inorganic fractions. From this result it follows that it is not the permeability of the corpuscles which is influenced by the presence of cyanide but the phosphorylation process occurring in the corpuscles.[11] Dische[24] has shown that under certain conditions in red corpuscles, in the presence of glucose and pyruvic acid, inorganic phosphate is esterified, the phosphorylation being inhibited by iodoacetic acid.

Potassium cyanide was found to obstruct the formation of labeled phytate and of labeled phosphatides in the nucleated corpuscles of the goose, as shown by Rapoport, Leva, and Guest[21] (Table 187). When labeled blood was incubated in an atmosphere of nitrogen, the formation of labeled phytate and labeled phosphatides was markedly reduced. The effects of incubation in an atmosphere of nitrogen for 1 to 3 hours were completely reversible, while the effects after 14-hour incubation were found to be partly reversible, only. As is seen in the table, the rate of renewal of phytate was found to be almost as low as the rate of

[23] S. Rapoport and G. M. Guest, *J. Biol. Chem.*, **138**, 269 (1941).

[24] Z. Dische, *Naturwissenschaften*, **24**, 462 (1936).

renewal of the phosphatides.   Thus, phytic acid, an acid-soluble compound of comparatively small molecular weight, falls in the class of the

TABLE 186

Uptake of ^{32}P by Rabbit Corpuscles at 37° after Treatment
with Cyanide Solution[11]

KCN added per ml. blood, mg.	Shaken previous to ^{32}P addition, min.	Shaken after ^{32}P addition, min.	[32]P uptake by corpuscles, %	[32]P distribution between P fractions of corpuscles, %	
				Inorganic	Organic
0		97	23.8	30	70
0		101	25.5	34	66
0		105	22.0	19	81
0.1	78	95	17.5	52	48
1.5	22	64	16.8		
1.5	92	101	13.5	91	9
2.0	155	102	15.6	60	40
2.0	51	97	12.8	56	44

phosphorus compounds renewed at a slow rate.   The presence of phytic acid in the blood of birds and turtles was observed by Rapoport[25]; the

TABLE 187

Formation of Labeled Compounds in Red Corpuscles of the Goose[21]

Conditions	Incubation time, hr.	Radioactivity per mg. P compared with that of acid-soluble P other than phytate P	
		Phytate	Phosphatide
O$_2$	24	2.72	1.79
KCN	24	0.03	0.08
N$_2$	24	0.019	0.008
O$_2$ (after 3-hr. incubation in N$_2$)	12	1.29	1.50

amount of phytate phosphorus in the red corpuscles was as large as or larger than all other acid-soluble phosphorus.

F. RATE OF RENEWAL OF PHOSPHATIDES

In Table 188 it is shown that the rate of renewal of the phosphatides in the corpuscles is a very slow process; here, the relative specific activities of different phosphorus fractions are shown 4 and 12 hours after subcutaneous injections of labeled phosphate into rabbits.   When

[25] S. Rapoport, *J. Biol. Chem.*, **135**, 403 (1940).

interpreting the figures in the table, it must be considered that at least some of the labeled phosphatides found in the corpuscles were not synthesized in the corpuscles, but were taken up from the plasma, and their origin may be traced to the liver (see page 306).

TABLE 188

Specific Activity of Phosphatide and Acid-Soluble Phosphorus of Corpuscles[26]

Fraction	Relative specific activity after	
	4 hr.	12 hr.
Phosphatide P	2.6	9.6
Inorganic P.................................	100	100
Pyrophosphate P..............................	99.5	100
Hydrolyzed P, 1 N H_2SO_4, 7–100 min............	100	
Hydrolyzed P, 100 min.–12 hr..................	100	100
Nonhydrolyzed P	87	

The plasma origin of some corpuscular phosphatides is shown in the finding that when rabbit corpuscles are shaken with plasma containing labeled phosphatides, a partition of some of the labeled phosphatides takes place. This partition becomes clear from Table 189.[26] Furthermore, 5 hours after injecting plasma containing labeled phosphatides into the circulation of the dog, 1.5 to 1.15% of the labeled phosphatides was found in the red corpuscles.[27]

The phosphatide molecules situated in the outermost layer of the stroma presumably participate in this exchange process, which is greatly facilitated by the presence in the stroma of a very large part of the phosphatides of the corpuscles [28,29] and by the monomolecular[28] or bimolecular[30] layer of phosphatides covering the corpuscle surface. The percentage of labeled phosphatides in the corpuscles which was taken up from the plasma is not negligible. The specific activity of the corpuscle phosphatides, 28 hours after administering labeled phosphate to a

[26] G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **15**, No. 5 (1940).

[27] D. B. Zilversmit, C. Entenman, M. C. Fishler, and I. L. Chaikoff, *J. Gen. Physiol.*, **26**, 3303 (1943).

[28] B. N. Erickson, H. H. Williams, S. S. Bernstein, I. Arvin, R. L. Jones, and I. G. Macy, *J. Biol. Chem.*, **122**, 515 (1938).

[29] F. O. Schmitt, R. S. Bear, and E. Ponder, *J. Cellular Comp. Physiol.*, **11**, 309 (1938).

[30] D. G. Dervichian and M. Macheboeuf, *Compt. rend.*, **206**, 1511 (1938).

.hen, was found to be only one-third that of the plasma phosphatides.[31]

In experiments *in vitro*, the formation of minute amounts of labeled phosphatides only was found to take place.[12a]

TABLE 189

In Vitro[a] Partition of Labeled Phosphatides, Originally in Plasma, between Phosphatides of Corpuscles and of Plasma[26]

Time, hr.		Extent of partition, %
0.5	....	1.8
1.5	....	3.6
3.0	....	4.0
4.5	....	5.0

[a] Rabbit plasma containing labeled phosphatides shaken with corpuscles of another rabbit.

While phosphatide molecules can thus be renewed in the corpuscles and interchange between the phosphatide molecules of the stroma and the plasma presumably can also take place, appreciable release of phosphatides from the corpuscles to the plasma does not occur. This follows, for example, from the fact that in lactating cows during fasting a marked decrease in concentration of plasma phosphatides takes place, which persists for several weeks after fasting stops, but there is no significant change in amount of red corpuscle phosphatides.[32] Even following parturition, the lecithin present in the red corpuscles of the cattle remained within normal limits, the acid-soluble phosphorus content was found to be markedly decreased, however.

G. RATE OF RENEWAL OF NUCLEIC ACIDS

The amount of nucleic acid present in mammalian red corpuscles is insignificant, the nucleic acid phosphorus in the blood of the rabbit amounting to less than 1% of the total phosphorus content. In avian blood the corresponding figure is over 50. The quantitative determination of nucleic acid in blood encounters difficulties. Therefore, most authors just consider the difference between the total phosphorus content and the acid-soluble + phosphatide phosphorus content, the so-called residual phosphorus, to be nucleic acid phosphorus. It is not

[31] G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **14**, No. 2 (1938).

[32] J. A. Smith, *Biochem. J.*, **32**, 1856 (1938).

known to what extent the nucleic acid of the nucleated red corpuscles is composed of ribo- and desoxyribonucleic acid, respectively. This distinction is of great importance in renewal considerations, since the rate of renewal of ribonucleic acid differs appreciably from the rate of renewal of desoxyribonucleic acid (see page 334).

After shaking hen blood containing labeled phosphate in an oxygen atmosphere for twenty-four hours, no formation of labeled desoxyribonucleic acid was observed.[33] Since the rate of renewal of desoxyribonucleic acid in the corpuscles of the hen is negligible, the presence of radiophosphorus in desoxyribonucleic acid extracted from the avian corpuscles can be interpreted as being incorporated in the course of the formation of the corpuscles. We can thus determine the life cycle of avian corpuscles by comparing the activity of 1 mg. desoxyribonucleic acid phosphorus with the activity of 1 mg. inorganic plasma phosphate phosphorus, as described on page 480.

The specific activity of the residual phosphorus present in the red corpuscles of the goose, which is presumably composed of desoxyribonucleic acid phosphorus + other residual phosphorus, was found in the experiments *in vitro* to be of a magnitude similar to the specific activity of phosphatide phosphorus.[34] Assuming the absence of contamination by acid-soluble phosphorus this result, in view of the negligible rate of renewal of desoxyribonucleic acid, indicates a fairly high rate of renewal of ribonucleic acid phosphorus or of a possible protein phosphorus content of avian corpuscles.

III. Permeability of Red Corpuscles to Phosphate

A. GENERAL REMARKS

Rate of penetration of phosphate into the corpuscles can be determined by the usual chemical methods.[34a] By replacing part of the chloride of the plasma, for example, by phosphate, the phosphate concentration of the plasma increases; this in turn leads to an increase in the phosphate concentration of the corpuscles. However, we cannot determine the rate of phosphate interchange between corpuscles and plasma by any other method than by applying isotopic indicators. The rate of interchange is the most direct measure of the permeability of the phase boundary.

[33] G. Hevesy and J. Ottesen, *Nature*, **156**, 534 (1945).

[34] S. Rapoport, E. Leva, and G. M. Guest, *J. Biol. Chem.*, **139**, 633 (1941).

[34a] R. Z. Solomon, P. M. Hald, and J. H. Peters, *J. Biol. Chem.*, **132**, 723 (1940).

After adding 0.4 ml. phosphate buffer of physiological concentration to 20 ml. rabbit blood, 12.5% of the phosphorus atoms present at the start of the experiment in the plasma is found to be accumulated in the corpuscles after the lapse of 2.5 hours, as seen in Table 190. In the

TABLE 190

Distribution between Plasma and Corpuscles
of Sodium Phosphate Added to Blood[11]

	Acid-soluble P of plasma, milligram per cent	
Sample	At start	2.5 hr. later
1	12.20	10.66
2	12.12	10.72
3	12.67	10.74
4	12.30	10.73
5	12.16	10.70
6	12.21	10.73
7	12.25	10.80
8	12.28	10.73
9	12.27	10.73
Average	*12.27*	*10.73*

same time, 35% of the radiophosphate added simultaneously with the phosphate buffer penetrated the corpuscles; similar results were obtained in experiments with human blood. This migration of ^{32}P takes place even when no excess phosphate is added to the blood. It indicates an interchange of phosphate between plasma and corpuscles; ^{32}P atoms moving from the plasma to the corpuscles are replaced by ^{31}P atoms moving in the opposite direction. If one supposes that 35 active phosphate ions move from the plasma to the corpuscles, when the phosphate concentration of the plasma is raised only 22.5 (35 − 12.5 = 22.5) phosphate ions would diffuse in the opposite direction.

In the experiments of Eisenman and associates,[35] when the free phosphate concentration of human plasma was raised to about 50 times its normal value, the amount of ^{32}P and ^{31}P penetrating the corpuscles in the course of 4 hours was found to be 44 and 27%, respectively. Values almost identical to these were obtained in experiments in which the phosphate concentration of the plasma was only slightly raised.

[35] A. J. Eisenman, L. Ott, P. K. Smith, and A. W. Winkler, *J. Biol. Chem.,* **135,** 165 (1940).

In experiments of restricted duration it is a point of great interest that the percentage chance of entrance of radioactive phosphate ions into the corpuscles is greater than the chance of their percentage return to the plasma. This is due to the fact that the labeled phosphate ions, within a short time after their entrance, participate in phosphorylation processes going on in the corpuscles and are thereby removed from the inorganic phosphate fraction. Therefore, the percentage chance that ^{32}P atoms move from the corpuscles to the plasma is appreciably smaller than that of movement in the opposite direction. Consequently, the ^{32}P content of the corpuscles remains practically constant for some time, making possible the use of such labeled corpuscles in the determination of the volume of the circulating blood and in other investigations, as discussed on page 469.

Bayard [36] has found that the addition of sodium arsenate to rabbit blood did not influence the rate of penetration of ^{32}P into the corpuscles; however, the addition of both sodium arsenite and potassium cyanide has a blocking effect.

B. PENETRATION OF PHOSPHATE INTO CORPUSCLES OF HUMAN SUBJECTS

Labeled phosphate was found to penetrate human corpuscles at a similar rate as into rabbit corpuscles. In experiments carried out *in vitro* at 37° C., after the lapse of 95 minutes, 29.2 to 31.7% of the labeled phosphate ions added to the blood was found in the corpuscles.[11] In experiments lasting 4 hours at 38° C., values varying between 27 and 44% are reported.[37]

Labeled human corpuscles, when shaken with inactive plasma at 37° C., were found to lose, in the course of 2 hours, 2–3% of their ^{32}P content.

The rate of penetration of radiophosphate into the red corpuscles of the rabbit, man, and horse is shown in Fig. 84.[38] The permeability of the red corpuscles of the horse is found to be very much lower than that of rabbit or of human corpuscles. The permeability of red corpus-

[36] P. Bayard, *Bull. soc. roy. sci. Liége*, **11**, 620 (1942).

[37] P. K. Smith, A. J. Eisenman, and A. W. Winkler, *J. Biol. Chem.*, **141**, 555 (1941).

[38] H. Levi, *Kgl. Danske Videnskab. Selskab Mat. fys. Medd.*, **23**, No. 10 (1945).
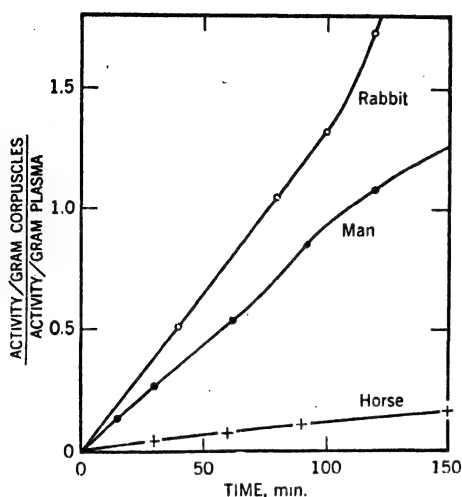
Fig. 84. Distribution of ^{32}P between red corpuscles and plasma of horse, man, and rabbit.[38] Horse corpuscles, and those of the hen (not given in the figure), show very low permeability.
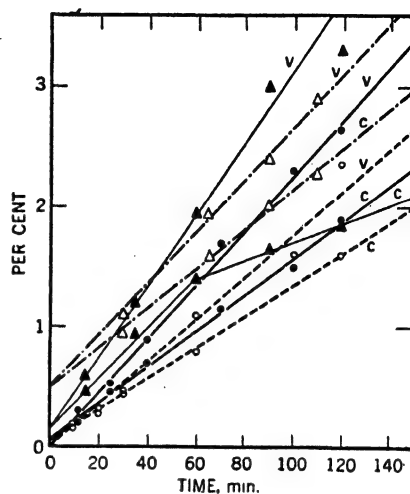
Fig. 85. Labeled phosphorus leaving the corpuscles.[39] Each pair of curves represents the amount entering the plasma with (v) and without (c) addition of bee venom, respectively.[39]

cles to phosphate ions was found by Levi[39] to be slightly increased by the action of bee venom, as seen in Figure 85.

TABLE 191

^{32}P Distribution *In Vitro* at 37° C. between Plasma and Red Corpuscles of the Hen[11]

Time, hr.	^{32}P found in corpuscles, %	Distribution coefficient of ^{32}P between corpuscles and plasma of equal weight
1	6.05	0.14
3.3	10.8	0.26
2.2	4.2	0.15
2.2[a]	3.2	0.11
1.7	5.0	0.12
4.7	6.3	0.23

[a] Corpuscles washed with sodium chloride solution.

[39] H. Levi, *Svenska Vetenskapsakad. Arkiv Kemi*, **A21**, No. 5 (1945).

C. PENETRATION OF PHOSPHATE INTO NUCLEATED CORPUSCLES

The rate of interchange between plasma phosphate and corpuscle phosphate was found to be much slower in hen blood than in bloods (with the exception of horse blood) containing nonnucleated erythrocytes. The rate of renewal of the organic phosphorus compounds was also found to be slower in the corpuscles of the hen. The distribution of ^{32}P between plasma and corpuscles of the hen is seen in Table 191. Phosphorus atoms in the nucleated erythrocytes of the frog were found to be replaced at a very slow rate, as is seen in Table 192.

TABLE 192

In Vitro Distribution of ^{32}P between Plasma and Corpuscles of the Frog[40]

Time, hr.	Temperature, °C.	Distribution coefficient of ^{32}P between corpuscles and plasma of equal weight
10	15	0.28
14	20	1.10
45	20	3.60

D. PENETRATION OF HEXOSE MONOPHOSPHATE

In the above discussion we assumed that it is the labeled phosphate ion which penetrates the corpuscle where it is largely incorporated into organic compounds, and that the molecules of the organic phosphorus compounds do not penetrate or penetrate the corpuscle membrane at a negligible rate. The validity of this assumption was tested in the case of hexose monophosphate.[10] Labeled hexose monophosphate was added to a blood sample. Part of the labeled hexose monophosphate was hydrolyzed during the experiment, labeled phosphate being split off. The values of the corpuscle activity measured after the lapse of 1–3 hours were compared with the values calculated according to the assumption that the active hexose monophosphate does not participate in the penetration process. These calculated and the experimental values are shown in Table 193. If labeled hexose monophosphate could penetrate the corpuscles, very different values from those recorded in the table would have been found.

The percentage of labeled hexose monophosphate decomposed in 175

[40] G. Hevesy, L. Hahn, and O. Rebbe, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **16**, No. 8 (1941).

minutes, when hexose monophosphate containing 1/15 mg. phosphorus was added to 10 ml. of rabbit blood, was found to be 31.

TABLE 193

Ratios of Specific Activities of Acid-Soluble ^{32}P in Red Corpuscles (S_c) and Plasma (S_p) of the Rabbit after Addition of Labeled Hexose Monophosphate[10]

Time, min.	S_c/S_p values	
	Calculated	Found
80	0.04	0.03
80	0.04	0.04
170	0.07	0.07
170	0.07	0.09

It is highly probable that the phosphorus atoms present in most acid-soluble organic phosphorus compounds of the red corpuscles reached the erythrocytes as inorganic phosphate ions from the plasma and were incorporated into organic molecules inside the corpuscles. The possibility that simultaneously with this process a slow exchange of, for example, organic phosphoglycerate between plasma and corpuscles takes place cannot be disregarded. In view of the low content of organic acid-soluble phosphorus compounds in the plasma, if migration of these compounds between corpuscles and plasma would take place, it would be directed mainly from the corpuscles into the plasma. In view of the rapid rate of renewal going on in the corpuscles and the rapid renewal of the acid-soluble phosphorus compounds in the plasma, the investigation of a possible migration of organic acid-soluble phosphorus molecules from the corpuscles into the plasma, or vice versa, encounters difficulties.

E. EFFECT OF TEMPERATURE ON RATE OF PENETRATION OF PHOSPHATE INTO RED CORPUSCLES

Early workers (Ege and Iversen[41]) showed that the corpuscle membrane of human and rabbit blood is only slightly permeable to phosphate at 3° C. and that this permeability increases somewhat with

[41] R. Ege, *Studier over Glukosens Fordeling mellem Plasmaet og de røde Blodlegemer*, Busck, Copenhagen, 1919. P. Iversen, *Biochem. Z.*, **114**, 297 (1921).

rise in temperature.   Halpern[42] later found that phosphate does not diffuse across the corpuscle membrane at 3° C. and diffuses only at a very slow rate at 23°; at 37.5°, however, the rate of diffusion is greatly accelerated.   Penetration of phosphate into the corpuscles was tested by noting whether inorganic phosphate added to the blood would enter the corpuscles or whether it would be carried out of the corpuscles with water after addition of hypertonic sodium chloride or sucrose solutions to the blood.

The application of radiophosphorus in permeability studies shows that the amount of phosphate penetrating the corpuscle membrane is much smaller at 0° C. than at 37° C., the permeability being, however, easily determinable even at 0°.   As is seen in Table 194, by increasing the temperature from 0° to 37° C. the rate of penetration of phosphate into the corpuscles increases 16 to 20 times.

TABLE 194

Effect of Temperature on Rate of Penetration of ^{32}P into Rabbit Corpuscles[11]

Time	Temperature, °C.	Per cent ^{32}P added to blood present in corpuscles
64 min.	0	1.29
66	37	25.9
72	0	1.24
72	37	19.3
7 hr.	0	14.6
7	0	12.7

The equation for diffusion rate is $D = ASe^{-Q/RT}$.   In this equation, A denotes the number of collisions, S the probability of energetically satisfactory encounters, Q the energy of activation, R the gas constant, and T the absolute temperature. Q is calculated to be 15,000 cal.

Eisenman and colleagues[35] in 4-hour experiments found that the rate of penetration of ^{32}P in human red corpuscles was about 30 times larger at 38° than at 7° C., while the amount of phosphate accumulating in the corpuscles when the phosphate concentration of the blood was increased was found to be only 4 times as large at 38° as at 0° C.

In the accumulation of ^{32}P of negligible weight in the corpuscles the incorporation of these atoms into organic phosphorus compounds is involved, the entrance of ^{32}P being compensated by the release of ^{31}P.

[42] L. Halpern, *J. Biol. Chem.*, **114**, 747 (1936).

The additional accumulation of phosphate in the corpuscles is due mainly to a replacement of chloride or bicarbonate ions of the corpuscles by phosphate of the plasma. The above results suggest that the temperature coefficient of this replacement differs widely from the coefficient of phosphate interchange.

IV. Retention of Radiophosphorus by Human Blood

The percentage of labeled phosphate of appreciable activity (15 millicuries), administered to human subjects, which was found by Erf and Lawrence[43] in 100 ml. of plasma, erythrocytes, leucocytes, and total blood in experiments of 2 to 192 hours' duration is shown in Table 195 (see also Erf et al.[44,45]).

TABLE 195

Percentage of Orally Administered ^{32}P in 100 Milliliters of Plasma, Erythrocytes, Leucocytes, and Whole Blood of Humans[43]

Time, hr.	Plasma	Erythrocytes	Leucocytes	Whole blood
2	0.0613	0.0533	0.0580	0.0606
6	0.0160	0.0873	0.0086	0.068
24	0.0106	0.0913	0.0266	0.047
48	0.0113	0.0647	0.0332	0.040
96	0.0100	0.0320	0.0693	0.025
192	0.0033	0.0273	0.0600	0.010
Average values for 3 other subjects				
2	0.082	0.1433	0.0693	0.1280
6	0.0260	0.1753	0.0600	0.1373
24	0.0215	0.1660	0.0873	0.1000
48	0.0215	0.1133	0.2460	0.0853
96	0.0215	0.0533	0.2126	0.0593
192	0.0126	0.0326	0.2270	0.0300

The ^{32}P content of the plasma reaches its maximum between 2 and 6 hours after *oral* administration of 600 mg. labeled sodium phosphate to subjects kept on normal diet; the peak for erythrocytes occurs between 6 and 24 hours, for the leucocytes between 48 and 96 hours. The whole

[43] L. A. Erf and J. H. Lawrence, *Ann. Internal Med.*, **15**, 276 (1941).

[44] L. A. Erf and J. H. Lawrence, *J. Clin. Invest.*, **20**, 567 (1941).

[45] L. A. Erf, L. W. Tuttle, and J. H. Lawrence, *Ann. Internal Med.*, **15**, 487 (1941).

TABLE 197

Distribution of Radioactive Phosphorus in Leukemic Blood[48]

Days after admin.	Leucocytes			Erythrocytes			Whole Blood			Plasma		
	% dose/ 100 ml.	mg. P/ 100 ml.	μc./ 100 mg. P	% dose/ 100 ml.	mg. P/ 100 ml.	μc./ 100 mg. P	% dose/ 100 ml.	mg. P/ 100 ml.	μc./ 100 mg. P	% dose/ 100 ml.	mg. P/ 100 ml.	μc./ 100 mg. P
Case I												
2	0.230	234	2.94	0.148	80.5	5.50				0.021	12.2	5.22
4	0.214	226	2.82	0.105	83.0	3.78	0.061	54.0	3.39	0.015	12.6	3.42
9	0.212	206	3.04	0.053	84.0	1.90	0.047	53.8	2.61	0.012	12.5	2.96
14	0.253	204	2.69	0.053	85.0	1.87	0.044	54.7	2.36	0.0094	12.4	2.25
53	0.136	227	1.79	0.029	70.7	1.24	0.022	52.0	1.26	0.0075	15.0	1.51
Case II												
0.5	0.20	261	3.58	0.220	64.5	15.8	0.125	64.5	9.11	0.023	16.8	6.30
2	0.23	262	4.17	0.110	75.0	6.94	0.078			0.021	20.0	4.90
4	0.29	257	5.53	0.065	72.5	4.18	0.060	62.2	4.52	0.015	17.3	4.21
9	0.30			0.049	72.5	3.21	0.059	67.6	4.08	0.0115	16.9	3.22
14	0.24			0.042	70.0	2.80	0.043	48.5	4.17	0.0088	16.2	2.58

[48] L. W. Tuttle, K. G. Scott, and J. H. Lawrence, *Proc. Soc. Exptl. Biol. Med.*, **41**, 20 (1939).

blood has the highest activity between 6 and 24 hours. Different results are obtained after *intravenous* administration of the labeled phosphate, as seen in Table 196. The ^{32}P of the hemoglobin fraction and of the stroma of the erythrocytes was determined as well. One day after intravenous injection no ^{32}P was found in the stroma; in 4 days the stroma contained 0.6% of the ^{32}P of the hemoglobin fraction. Twenty-one days after oral administration the corresponding figure was found to be 0.9. The increase in ^{32}P of the stroma with time is presumably due to increased labeling of the stroma phosphatides.

Red corpuscles took up more ^{32}P per 100 ml. in the first days of the experiment than leucocytes, the opposite being the case in the later phases of the experiment. ^{32}P is taken up by the corpuscles partly by

TABLE 196

Percentage[a] of Intravenously Administered ^{32}P in 100 Milliliters of Human Plasma, Erythrocytes, Leucocytes, and Whole Blood[43]

Time, hr.	Plasma	Erythrocytes	Leucocytes	Whole blood
2	0.0873	0.2266	0.1822	0.1733
6	0.0186	0.2346	0.0733	0.1713
24	0.0240	0.1886	0.0753	0.1120
96	0.0173	0.0780	0.1120	0.0220
192	0.0080	0.0470	0.1330	0.0280

[a] Average for 4 subjects.

interchange and partly by incorporation during the formation of the corpuscles in the labeled organism. The increased ^{32}P uptake by the leucocytes after the lapse of 24 hours is possibly mainly due to the formation of new leucocytes.

V. Distribution of Radiophosphorus in Leukemic Blood

Lawrence and associates[45-47] studied the uptake of ^{32}P in the red and white corpuscles of leukemic patients (see Table 197). It was observed that higher concentrations of ^{32}P occur in the nucleoprotein fraction of the leukemic leucocytes than in this fraction of normal leucocytes during a period of one week following administration, presumably due to more rapid formation of leukemic leucocytes. The

[46] L. W. Tuttle, K. G. Scott, and J. H. Lawrence, *Proc. Soc. Exptl. Biol. Med.*, **41**, 20 (1939).

[47] J. H. Lawrence, L. A. Erf, and L. W. Tuttle, *J. Applied Phys.*, **12**, 333 (1941).

finding that the difference between the nonnucleoprotein fractions of the normal and the leukemic leucocytes is smaller than the corresponding difference between the nucleoprotein fractions must be explained by the fact that the first-named fractions, in contradistinction to the nucleoprotein fractions, mainly take up ^{32}P during their renewal.

TABLE 198

Retention of Radiophosphorus in the Phosphatide, Acid-Soluble
and Residual (Nucleoprotein) Fractions of Leucocytes
of a Lymphoid Leukemia Patient[44]

	Radiophosphorus retention (μc./ml.) in leucocyte fraction		
Hours after admin.	Phosphatide	Acid-soluble	Residual
5 μc. oral			
12	0.0048	0.0465	0.0081
24	0.0099	0.0435	0.0111
48	0.0162	0.0417	0.0147
96	0.0228	0.0187	0.0393
2 μc. intravenous			
96	0.0068	0.0079	0.0248

The distribution of radiophosphorus in the different fractions of the leukemic white corpuscles is shown in Table 198. The phosphatide and the residual fractions reach their highest ^{32}P content after the lapse of

TABLE 199

Distribution of Subcutaneously Injected ^{32}P between Acid-Soluble
and Phosphatide Phosphorus of Plasma, Erythrocytes, and Leucocytes[43]

	Relative ^{32}P content					
Time	Plasma		Erythrocytes		Leucocytes	
	Acid-soluble P	Phosphatide P	Acid-soluble P	Phosphatide P	Acid-soluble P	Phosphatide P
24 hrs.	100	61	118	23	83	23
48	176	100	104	59	107	12
96	100	133	96	44	101	6
21 days	22	83	15	66	28	23

96 hours only, while the maximum activity of the acid-soluble fraction is already reached in 24 hours. A more detailed study of the phosphate permeability of the white corpuscles is still to be made.

The ^{32}P content of the acid-soluble and phosphatide fractions of the plasma, the erythrocytes, and the leucocytes was also determined by Lawrence and co-workers. In these experiments, 2.6 to 6 millicuries sodium phosphate was administered to patients suffering from polycythemia. The result obtained after subcutaneous injection is seen in Table 199.

VI. Application of Red Corpuscles Containing Radiophosphorus

A. GENERAL REMARKS

As described on page 460, corpuscles can be labeled with radiophosphorus, and these corpuscles can be used in the determination of the amount of circulating red corpuscles and also to solve numerous other problems.

B. DETERMINATION OF CIRCULATING RED CELL VOLUME

The amount of circulating red corpuscles is usually calculated from the amount of circulating plasma determined by the dye method and from the hematocrit figure. This method has various shortcomings,[49-50d] and it is important to have a direct method for determining the amount of circulating corpuscles. With ^{32}P labeled red corpuscles were first applied in the determination of the corpuscle content of circulating rabbit blood,[51-53] and later in the determination of the corpuscle content of the human circulation. The method described was also applied to the determination of the corpuscle content of the dog.[54]

[49] G. Nylin, *Svenska Vetenskapskad. Arkiv Kemi*, **A20**, No. 17 (1945).

[50] M. A. Chapin and J. F. Ross, *Am. J. Physiol.*, **137**, 447 (1942).

[50a] R. S. Anderson, *Am. J. Physiol.*, **137**, 539 (1942).

[50b] G. Nylin and S. Hedlund, *Am. Heart J.*, **33**, 770 (1947).

[50c] G. R. Meneely, E. B. Wells, and P. F. Hahn, *Am. J. Physiol.*, **148**, 531 (1947).

[50d] J. G. Gibson, W. C. Peacock, A. M. Seligman, and T. Sack, *J. Clin. Invest.*, **25**, 838 (1946).

[51] G. Hevesy and K. Zerahn, *Acta Physiol. Scand.*, **4**, 376 (1942).

[52] L. Hahn and G. Hevesy, *Acta Physiol. Scand.*, **1**, 3 (1940).

[53] R. S. Anderson, *Am. J. Physiol.*, **137**, 539 (1942).

[54] J. Govaerts, *Acta biologica belgica*, **4**, 425 (1942).

Generally, blood containing both labeled corpuscles and labeled plasma is injected, and after the lapse of 10 minutes, for example, a blood sample is secured. Let us denote the amount of corpuscles injected into the rabbit by A, the ratio of activity of 1 g. corpuscles of the blood injected and of 1 g. corpuscles secured from the circulation after the injection by B; then the total amount of corpuscles present in the circulation (X) is given by the equation $X = AB$. The main sources of error of the method are that some plasma adheres to the centrifuged corpuscles, that the injected plasma contains radiophosphorus, that some ^{32}P enters the corpuscles in the course of the experiment, and that some of the ^{32}P present in the injected corpuscles is released in the course of the experiment. The magnitude of these errors in experiments on rabbits is shown in Table 200.

TABLE 200

Estimate of Experimental Errors in Determination
of Circulating Corpuscles of the Rabbit[51]

Minutes after injecting labeled corpuscles	Percentage error due to		
	Adherence of plasma to corpuscles	^{32}P entrance into corpuscles	^{32}P loss by corpuscles
6	+1.6	−3.4	+1
12	+1.8	−3.9	+1.5

The errors compensate for each other to a large extent. A removal of the active plasma before injection is unnecessary, and not even advisable. This is also seen from results obtained by Nylin[49] who determined the circulating red corpuscle volume of human subjects, by injecting labeled blood and then labeled corpuscles alone. The results obtained are shown in Figure 86.

In experiments on human subjects carried out by Nylin,[49,55,55a] Hevesy et al.,[56] Govaerts,[54] and Hedlund, labeled phosphate of negligible weight, having an activity of 1 microcurie or preferably several microcuries is dissolved in a small volume (0.1 ml.) of physiological sodium chloride solution, is added to about 10 ml. of the subject's blood. After gently shaking the sample at 37° C. for 1 to 2 hours, an aliquot of

[55] G. Nylin and M. Malm, *Cardiologia*, **7**, 153 (1943); *Am. J. Med. Sci.*, **207**, 743 (1944).
[55a] G. Nylin, *Brit. Heart J.*, **7**, 81 (1945); *Am. Heart J.*, **34**, 174 (1947).

the labeled blood is reinjected into the human subject. At appropriate intervals, blood samples are secured, centrifuged, and the activity of the dried corpuscles determined (see page 46). An alternative method is to dissolve the corpuscles by wet ashing and to precipitate an aliquot,
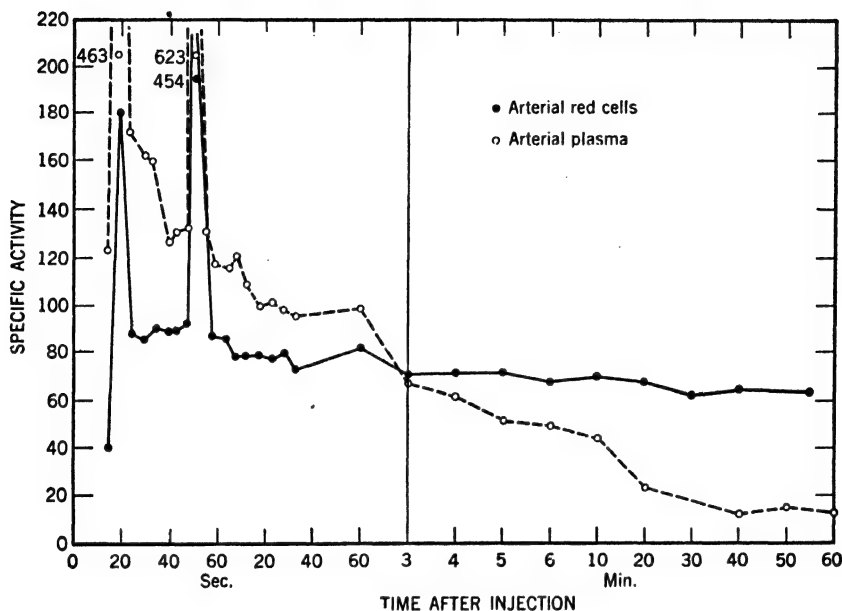
Fig. 86. Specific activity of both corpuscles and plasma after intravenous injection of labeled blood.[49]

after adding 40 mg. inactive sodium phosphate, as magnesium ammonium phosphate. The activity of the corpuscle samples secured is then compared with the corresponding value of the injected corpuscles. Corpuscle samples having an activity of 50 counts per minute can be measured conveniently. The time of measurement can be shortened appreciably when disposing samples over 10 times as active as stated above. Figure 86 shows the change in activity of the corpuscles and the plasma. About 3 minutes after the labeled blood was injected, as seen in Figure 87, the activity of 1 g. of corpuscles reached a constant level, i.e., after the lapse of this time a homogeneous mixing of the injected labeled corpuscles with the circulating corpuscles is obtained in normal subjects[49] (see also Hevesy et al.[56]). Nylin and Hedlund

[56] G. Hevesy, K. H. Köster, G. Sörensen, E. Warburg, and K. Zerahn, Acta Med. Scand., 116, 561 (1944).

found the ^{32}P content of reinjected corpuscles to remain within the errors of the experiments (4%) unchanged for at least 60 minutes.[56a]

Nylin's very extended investigations[49] include among others a study of the effect of the injection of adrenaline, of severe muscular exercise, of the effect of latent shock and postural hypotension on the circulating red corpuscle volume. He also determined the circulating corpuscle volume of the lungs and the legs.
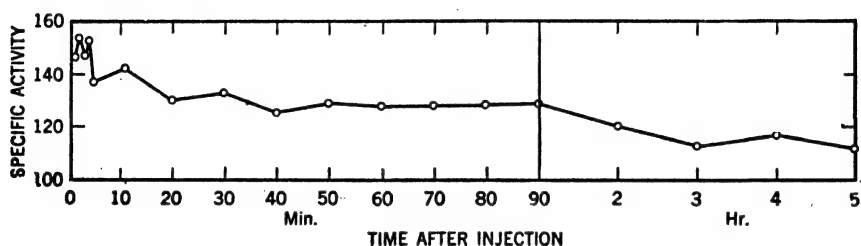
Fig. 87.   Specific activity of corpuscles after intravenous injection of only labeled corpuscles.[49]

In healthy persons no appreciable increase in the circulating blood volume (calculated from the determined corpuscle volume and the hematocrit value) was observed due to muscular exercise. In order to study the effect of muscular exercise on the circulating corpuscle volume, the greatest long-distance runner of Sweden was made to run 50 rounds on Nylin's stairs at a rate of 208 steps per minute after his circulating blood volume had been determined during rest. In doing this he raised and lowered his own weight 100 meters in the course of about 5 minutes. A repeated determination of the activity was made on venous blood, and the blood volume was calculated. The circulating blood volume was found to be increased during work by about 4%. In other cases a still smaller increase was found.[56b] There is thus no reservoir in man which empties red corpuscles into the blood stream after work, or, if there is, it is too small to be shown statistically.[56b]

Nor was the administration of adrenaline found to be followed by a dilution of the labeled corpuscles injected into the circulation due to release of nonlabeled corpuscles. No blood depots were thus emptied under the action of adrenaline.[56c]

[56a] G. Nylin and S. Hedlund, *Am. Heart J.*, **33**, 770 (1947).
[56b] G. Nylin, *Am. J. Physiol.*, **149**, 180 (1947).
[56c] G. Nylin, *Acta Cardiol.*, **1**, 225 (1946).

C. RED CORPUSCLE CONTENT OF THE LEGS

The vessels of the legs were clamped with blood pressure cuffs in which the pressure was elevated above the systolic blood pressure of the legs. Labeled corpuscles were then injected into the circulation and, from the activity of a corpuscle sample secured after the lapse of 12 minutes (see Figure 88), the circulating red corpuscle volume was calculated. The next step was to remove the blood pressure cuffs, which led to a decrease in activity of the circulating blood due to the

Red blood corpuscles	1560 g.	23 g./kg.	1445 ml.	22 ml./kg.
Plasma	2410	36	2360	35
Circulating blood vol.	3970	59	3805	57

Circulation to the legs shut off

Circulation to the legs released

Red blood corpuscles	1810 g.	27 g./kg.	1645 ml.	25 ml./kg.
Plasma	2790	42	2730	41
Circulating blood vol.	4600	69	4405	66
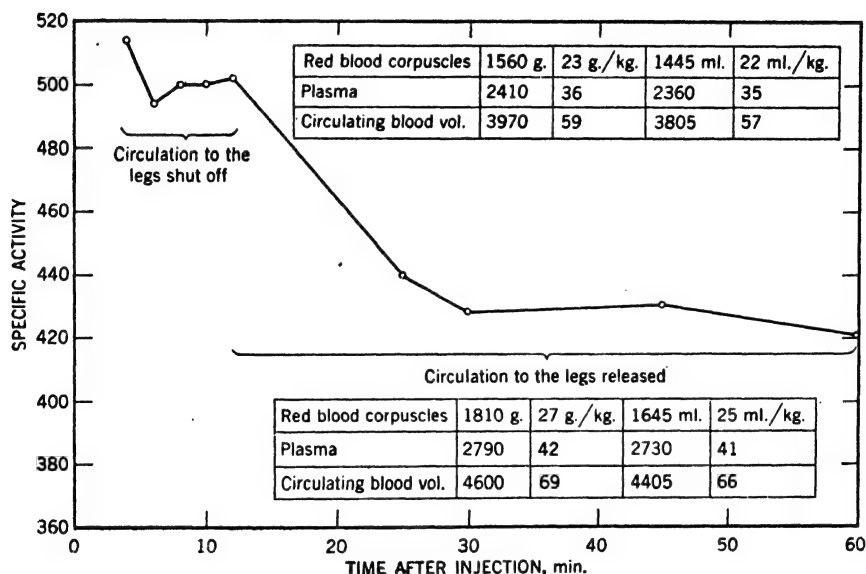
Fig. 88. Determination of the circulating corpuscle content of the legs.[49]

influx of the inactive leg blood into the circulation. The amount of circulating red corpuscles was now found to be increased from 1560 to 1810 g. From these figures it follows that the red corpuscle content of the legs amounts to 250 g.

D. RED CORPUSCLE CONTENT OF THE LUNG

The circulating red corpuscle content in one lung has been studied in cases of lung extirpation. During the preparation for pulmonectomy in a case of tumor of the lung, all the vessels of the diseased lung were clamped. The labeled blood corpuscles were then injected into the patient, and a series of specimens were taken to determine the circulatory blood volume up to 16 minutes after the injection had been given.

The circulation of the clamped lung was then freed, and a renewed series of specimens were taken. As appears from Figure 89, the activity fell after the lung circulation had been freed. In this case, the increase in the circulatory blood volume was 911 g., *i.e.*, 16.7%, which would thus be the blood volume of the left lung.[49,56d]
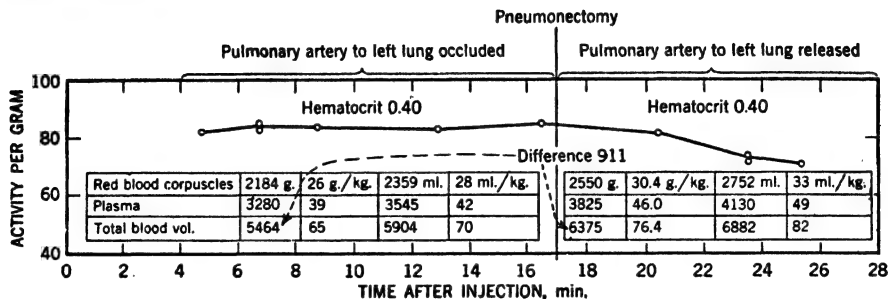
Fig. 89. Circulatory red corpuscle content of one lung.[49]

E. STUDY OF RESIDUAL BLOOD CONTENT OF THE HEART

Nylin[49,56e] used labeled red corpuscles in determining the relation between the amount of residual blood of the heart and the velocity of the blood flow. Labeled blood corpuscles were injected into the arm vein of a normal person in recumbent position, and immediately afterwards blood specimens were withdrawn from the brachial artery of the
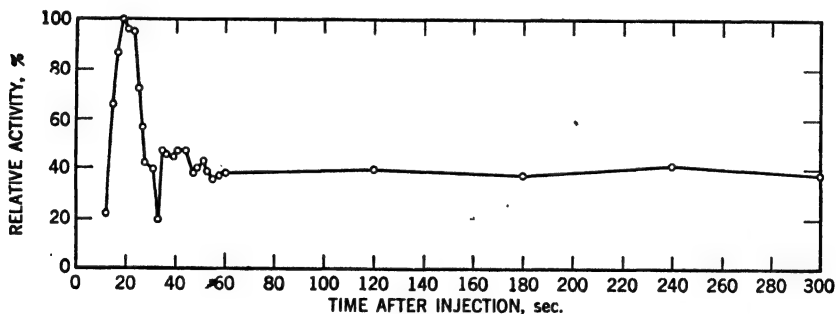
Fig. 90. Dilution curve in arterial blood after injection
of labeled blood corpuscles in a normal person.[49]

other arm. Subsequently, the activity of the blood specimens secured was determined and, in that way, a dilution curve of the type shown in Figure 90 was obtained.

[56d] G. Nylin, *Am. Heart J.*, **34**, 174 (1947).
[56e] G. Nylin, *Am. Heart J.*, **30**, 1 (1945).

While mixture of the injected blood with the circulating blood takes place within few minutes in normal subjects, in the case of heart insufficiency appreciable time may be necessary to attain such a state. In extreme cases, mixing is obtained only after the lapse of 1 hour, as is seen in Figure 91.
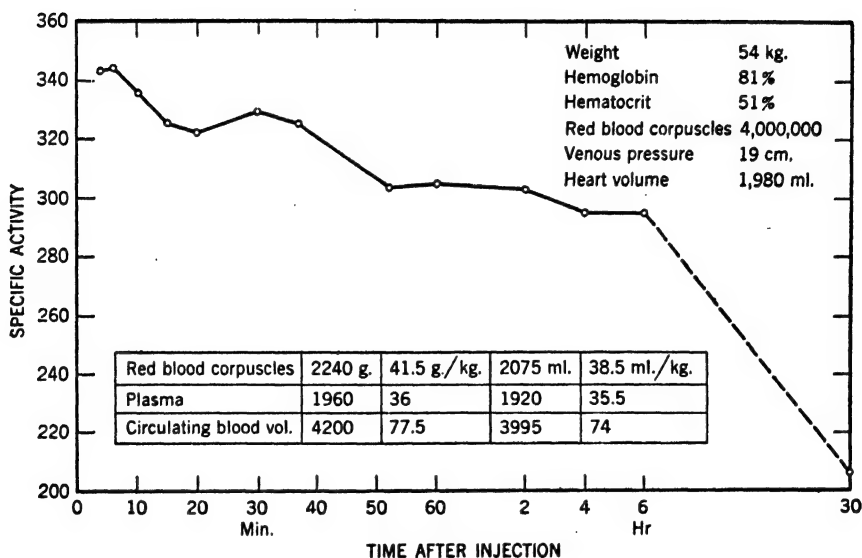
Fig. 91. Change of activity of circulating red corpuscles in a case of congestive heart failure.[49]

In cases of dilatation of the heart, with a large amount of residual blood in the heart, mixing with the indicator (labeled blood corpuscles) takes place considerably more slowly, as appears from Figure 92. Thus, the activity begins to increase in the arterial blood after 40 seconds, reaches a maximum at 70 seconds, and does not reach a constant level until 7 minutes after the injection. Subsequently, the activity is constant until at least the tenth minute. Estimation of the circulation time in cardiac patients with dilated hearts,[56f] but no peripheral edema, supports the view that blood is retained in dilated hearts and that this factor greatly influences the estimation of the circulation time. A comparison between the dilution curves in decompensated patients and normal subjects shows that the activity of the corpuscle samples secured is reduced slowly in decompensated patients. This seems to indicate

[56f] B. Gernandt and G. Nylin, *Am. Heart J.*, **32**, 411 (1946).

that the activated blood is kept for a certain time within the congested area.

In decompensated cardiac patients an increase in the amount of erythrocytes is found. After compensation is restored, both the plasma and blood corpuscles decrease in quantity. In decompensated patients
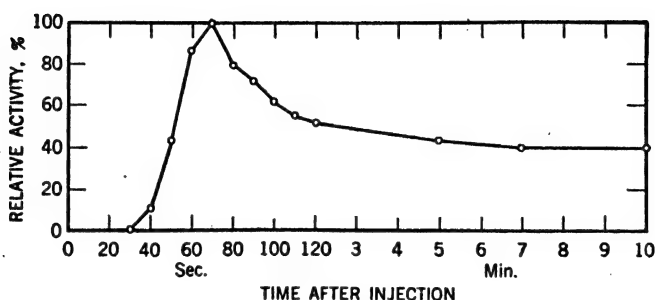
Fig. 92. Dilution curve in arterial blood after injection of labeled blood corpuscles in a case of heart dilatation.[49]

the quantity of erythrocytes is, on the average, 36.4 grams per kilogram of dry body weight; in normal subjects, this value is 31.2 grams per kilogram of dry body weight. About the same quantity of erythrocytes is found in compensated cardiac patients as is found in normal subjects.[56a]

The red corpuscle volume has been studied by means of the radio-active phosphorous method in a case of patent ductus arteriosus before and after operation. A decrease in circulating red corpuscle volume amounting to 8% has been found.[56g]

A phenomenon observed in some cases of congestive heart failure is the release of stored injected corpuscles (and blood), in a later stage of the experiment. Such a release is indicated by an increase in activity of circulating corpuscles, as seen in Figure 93. After the lapse of 50 minutes an apparent equilibrium is reached; the corpuscle activity calculated from this equilibrium is shown in the upper left text of the figure. The release of trapped corpuscles leads then to an increase in activity of circulating corpuscles and lower total corpuscle volume figures (see text in lower part of the figure) are now obtained.

In his studies on vascularization in the anterior horn of the medulla of rabbits, Krogh,[56h] using labeled red corpuscles, found that a very slow

[56g] G. Nylin and G. Biörck, *Acta Med. Scand.*, **127**, 434 (1947).
[56h] A. Krogh, *Acta Physiol. Scand.*, **10**, 271 (1945).

circulation took place in the anoxemic part of the medulla during occlusion. Exchange of blood in the capillaries, normally requiring less than a minute, now took an hour or more.

Red blood corpuscles	2820 g.	25.0 g./kg.	2610 ml.	23.5 ml./kg.
Plasma	4730	42.5	4640	42.0
Circulating blood vol.	7550	67.5	7250	65.5

Weight	111 kg.
Hemoglobin	70%
Hematocrit	36%
Red blood corpuscles	3,600,000
Venous pressure	13 cm.

Red blood corpuscles	2420 g.	22.0 g./kg.	2240 ml.	20.0 ml./kg.
Plasma	4060	36.5	3980	36.0
Circulating blood vol.	6480	58.5	6220	56.0
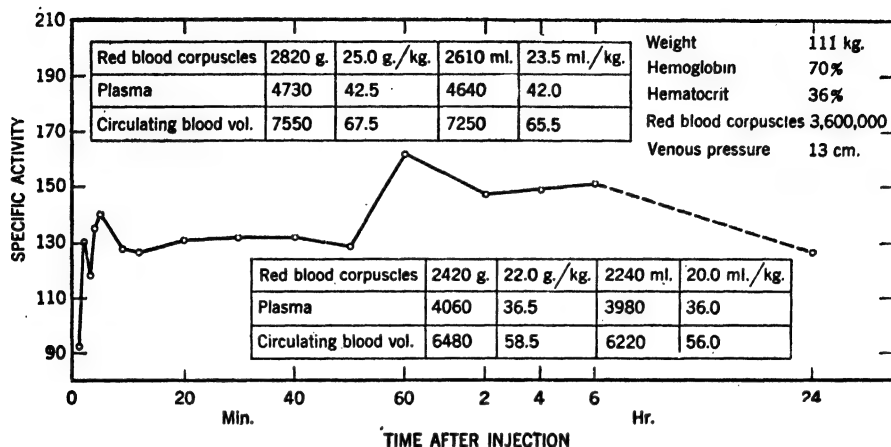
Fig. 93.   Change of red corpuscle activity with time
in a case of congestive heart failure.[56a]

F. EFFECT OF ORTHOSTATISM AND SHOCK ON CIRCULATION VELOCITY

Investigation of the velocity of mixing of the injected labeled red corpuscles with the circulating red corpuscles in standing and recumbent positions, and during shock induced by spinal anesthesia lead to the following results.[56i]

Mixing of the test substance and the blood takes place much later in standing than in the recumbent position; in the standing position the velocity of the blood flow is thus prolonged in some part of the body. In shock during spinal anesthesia, the mixing takes place very late and is accomplished only 75 minutes after the injection of the labeled corpuscles (in normal conditions the mixing is accomplished after 1 minute); the blood volume seems to be normal. Thus in shock conditions the changes in the velocity of the blood flow seem to be more important than the changes in blood volume.

G. PERMEABILITY OF THE PLACENTA

Red corpuscles labeled with radiophosphorus have been employed in the studies of the permeability of the placenta.[57] Labeled sodium

[56i] G. Nylin and R. Pannier, *Arch. intern. pharmacodynamie*, **73**, 401 (1947).

[57] J. Naeslund and G. Nylin, *Acta Med. Scand.*, Suppl. No. 170 (1946).

phosphate was mixed with heparin blood obtained from the arm vein of the pregnant woman a few hours prior to the expected parturition. The mixture was shaken repeatedly while contained in a thermostat at a temperature of 37° for 2 hours. The blood corpuscles were then decentrifugated, washed with a physiological sodium chloride solution, and placed in a refrigerator. Before the expected delivery, the blood corpuscles were mixed with heparinized inactive plasma from the woman, with the same relationship between the two as in her blood, whereupon 4 ml. were injected into the arm vein. Immediately after the birth of the child and the cutting of the umbilical cord, a blood sample was taken from this part after having been wiped clean from all traces of the mother's blood. Simultaneously or just afterward, a sample was also taken from the mother. Some of the blood samples from the mother and child were centrifugated as soon as possible, and the filterable components of the plasma were separated from the nonfilterable "protein substances and lipoids." This division was performed in order to make a distinction between the radioactive components in the plasma which might, conceivably, pass through the placenta by means of filtration, on the one hand, and the nonfilterable components, namely, "protein substances and lipoids," on the other.

TABLE 200A

Radioactivity[a] of Red Corpuscles and Plasma of Mother and Fetus[57]

Maternal blood			Fetal blood		
	Plasma			Plasma	
Blood corpuscles	Nonfilterable components	Filterable components	Blood corpuscles	Nonfilterable components	Filterable components
83	8	0	0	0	0
51	2	0	1	0	1
70	4	0	1	0	2
54	2	0	0	–	0
78	2	0	1	0	0
345	6	5	23	1	0

[a] In counts per gram per minute.

The results obtained are seen in Table 200A and indicate that radioactivity of both the maternal and the fetal plasma is insignificant. They show also in the first recorded five cases the radioactivity of the fetal corpuscles to be negligible. Not so, however, in the last stated case, which indicates that leakage in the placenta may, under certain condi-

tions, occur, causing the blood corpuscles to pass over from the mother to the fetus and probably also vice versa. This occurrence may, possibly, be responsible for the occurrence of pregnancy toxicosis and some other complications observed during pregnancy. All other results disclose the fact that in normal cases no labeled corpuscles pass through the placenta.

VII. Life Cycle of Red Corpuscles of the Hen

One would expect that the problem of the life of the red corpuscles could be solved easily by making use of an isotopic indicator, that is, by labeling the red corpuscles. In trying to find a suitable indicator, great difficulties are encountered due to the fact that almost every compound in the corpuscles is renewed at a comparatively rapid rate. Only such labeled molecules which have a longer half-life than the red corpuscles in which they are located can be used as indicators. Iron atoms incorporated with hemoglobin molecules remain unchanged during the lifetime of the red corpuscles. Hahn and colleagues,[58] however, found that the iron atoms contained in the debris of the hemoglobin of decayed corpuscles are preferentially used in the formation of new corpuscles. This fact makes radioactive iron unsuitable for the determination of the life cycle of red corpuscles (see page 503).

Desoxyribonucleic acid phosphorus was found to be a suitable indicator for the determination of the life cycle of nucleated corpuscles.[33] In contradistinction to the desoxyribonucleic acid molecules of various organs, those found in the red corpuscles of the hen are not renewed at an appreciable rate. In experiments *in vitro*, in which hen blood was shaken in an oxygen atmosphere in the presence of labeled sodium phosphate, active desoxyribonucleic acid, in contradistinction to other phosphorus compounds, was not formed. Furthermore, no activity was found in the desoxyribonucleic acid of the circulating red corpuscles of the hen up to five days after administration of radioactive phosphate.

Labeled phosphate is administered twice a day to the hen in such quantities that the plasma phosphate is kept at a constant or almost constant level of activity. The active phosphate penetrates the marrow and participates in the formation of the nucleic acid of the corpuscles, which thus become labeled. The percentage of labeled corpuscles will increase with time and, finally, the circulation will contain labeled corpuscles only; thus, the activity of 1 mg. corpuscle desoxyribonucleic

[58] P. F. Hahn, W. F. Bale, and W. M. Balfour, *Am. J. Physiol.*, **135**, 600 (1942).

acid phosphorus will be equal to the activity of 1 mg. marrow phosphorus and 1 mg. plasma phosphorus, respectively.

The results of such experiments are shown in Figure 94. It is clear from the figure that, in the first five days, the nucleic acid present in the corpuscles is inactive. This may be interpreted by assuming that, in the first phase of the experiment, corpuscles containing inactive nucleic acid reach the circulation, and that it is about five days before corpuscles containing labeled nucleic acid are given off by the sinusoids
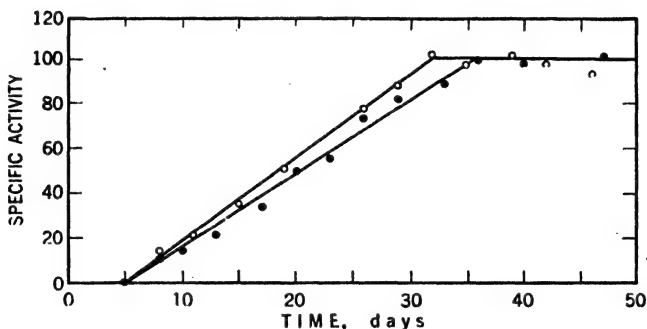
Fig. 94. Life cycle of the red corpuscles of two hens.[33] Ordinate: specific activity of desoxyribonucleic acid phosphorus extracted from the corpuscles secured at different dates.

to the circulation. The maturing of the corpuscles in the marrow thus takes about five days. The figure also shows that, after the lapse of about 33 days, the maximum value of the activity of the desoxyribonucleic acid is reached. Taking into account that in the first five days no labeled corpuscles reach the circulation, the lifetime of the red corpuscles will be 28 days. The results obtained indicate that all or almost all corpuscles present in the circulation have a similar lifetime.

Ottesen[59] obtained lifetime values almost identical with those stated above by the following simplified method. Labeled sodium phosphate is administered to the hen at the start of the experiment only. In such experiments, the initially high activity of the plasma is rapidly declining. Consequently, the erythrocytes formed during the first days of the experiment contain highly active desoxyribonucleic acid, while this compound present in corpuscles formed later has only restricted activity.

[59] J. Ottesen, *personal communication.* Recent observations reveal a maturing time of the red corpuscles amounting to only 2 days.

The radioactive desoxyribonucleic acid content of the circulation increases during the first 3 weeks, remains almost constant in the following few days, and then declines markedly. This decline is due to the fact that the first-formed, highly active corpuscles are now decaying. The lifetime of the red corpuscles can be computed from the date of rapid decline.

Mammalian erythrocytes do not contain appreciable amounts of phosphorus compounds having a longer lifetime than that of the red corpuscles. It is thus not possible to determine the life cycle of the mammalian red corpuscles by using ^{32}P as an indicator. Shemin and Rittenberg,[59a] however, found by feeding glycine labeled with ^{15}N to human subjects, that the heme of red corpuscles retains its ^{15}N content and concluded from their investigation that the erythrocyte is not subjected to indiscriminate destruction, but has a life span; thus the same result was obtained in the above-mentioned investigation of the life span of nucleated corpuscles. The life cycle of the human erythrocytes was however found by Shemin and Rittenberg to be about 127 days.

VIII. Arsenic and Sulfur

When 2 to 100 milligram per cent of radioarsenate was added to rabbit blood, 3–20% was found in the corpuscles after the lapse of 0.5 to 2 hours.[60] Even higher figures for the radioarsenic content of the corpuscles of the rat are found after subcutaneous injection of labeled sodium arsenite. The radioarsenic is attached to the hemoglobin molecule; on fractionation the globin portion yields twice as much as the heme fraction. No arsenic is found in association with the stroma of the cell.[61] Radioarsenic administered carrier free accumulates markedly in the red corpuscles (see page 129).

The rate of penetration of arsenate does not differ much from the rate of penetration of phosphate; this applies also to the rate of penetration of sulfate ion. Zerahn[62] found the coefficient of distribution of radiosulfate between corpuscles and plasma of the rabbit to be 0.30 after the lapse of 30 minutes, while, after 162 minutes, 0.45 was found. In these experiments, 0.05 ml. isotonic sodium sulfate was added to 1 ml. blood.

[59a] D. Shemin and D. Rittenberg, *J. Biol. Chem.*, **166**, 627 (1946).

[60] P. Bayard, *Bull. soc. roy. sci. Liége*, **11**, 620 (1942).

[61] F. T. Hunter and A. F. Kip, *J. Applied Phys.*, **12**, 324 (1941).

[62] K. Zerahn, *personal communication*.

IX. Halogens

The interchange between chloride and bicarbonate, which is intimately connected with the respiration process, is very rapid; in about two seconds half the equilibrium distribution is reached. The change in chloride concentration of the plasma is measured in these experiments by determining the change in potential of a silver chloride electrode.[63] In view of the slight resistance encountered by chloride ions when penetrating the corpuscle membrane, we can expect chloride ions to interchange speedily not only with bicarbonate ions, but also with other chloride ions. Labeled chloride ions introduced into the plasma should soon be found in the corpuscles, and vice versa.

Since separation of the corpuscles from the plasma takes some time, a physiological sodium chloride solution containing labeled chloride was added to rabbit blood at 0° C.; at this temperature the rate of penetration of chloride ions can be expected to be much slower than at 37° C. Even at 0° a large portion of corpuscle chloride was replaced by labeled chloride originally in the plasma in the course of 1–3 minutes.[11,60]

The distribution of chloride between corpuscle and serum was found by Weir and Hastings[64] to be 0.72, corresponding exactly to the value for the water ratio. The distribution coefficient of bromide was found to be larger (0.76), indicating that a minor part of the bromine of the corpuscles is in bound state (results obtained by Van Dyke and Hastings[65] indicate that even a certain proportion of the chloride of the corpuscles is present in nonionic form). Smith and Winkler[37] found a preferential uptake of labeled bromide by the corpuscles, the ratio of bromide in total halide of red corpuscles to that in total halide of serum being 1.4. (Cf. also Snyder et al.[2a]) The ratio of labeled iodide in total halide of corpuscles to that in total halide of serum is stated[37] to be 1.4.

Interesting results may also be obtained from the investigation of the labeled iodide content of the corpuscles after introducing labeled iodine into the circulation and studying the state in which iodine is present. In view of the small amounts involved, the application of radioactive indicators greatly facilitates such investigations. Although the bromine content of blood varies between 0.16 to 1.5 milligram per cent, the iodine content is very much lower, and the iodine content of

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[63] H. Luckner and Lo Sing, *Arch. ges. Physiol. Pflügers*, **239**, 278 (1938).

[64] E. G. Weir and A. B. Hastings, *J. Biol. Chem.*, **129**, 547 (1939).

[65] A. B. Hastings and H. B. Van Dyke, *J. Biol. Chem.*, **92**, 13 (1931).

the corpuscles is still uncertain.[66] Recently it was found by Joliot[67] that radioiodide is equally distributed between plasma and red corpuscles of the rabbit; but labeled thyroxine does not penetrate the corpuscles.

X. Sodium and Potassium

A. GENERAL REMARKS

The sodium content of the corpuscles (see Table 201) of most animals — cat and dog are exceptions — is much lower than the sodium content of the plasma; the opposite is true for potassium, the ratio of the potassium content of corpuscle water and that of plasma water reaching values up to 25. The large accumulation of sodium in the plasma and of potassium in the corpuscles, respectively, was interpreted previous to the application of isotopic indicators to be due to the impermeability of the corpuscle membrane to potassium and sodium ions.[68] It was supposed that potassium, for example, was concentrated in the corpuscles in the course of their formation, and the potassium atoms remained in these throughout their lifetime. The application of isotopic indicators has shown that radioactive sodium and potassium, respectively, present in the plasma penetrate the corpuscles, and vice versa.[69-74a] Impermeability of the corpuscle membrane to sodium and potassium can thus not be responsible for the accumulation of these ions in the plasma and the corpuscles, respectively. We have thus to find another explanation for the large differences in concentration of single ions inside and outside the corpuscles.

The mechanism responsible for this fact can be expected to resemble the mechanism which maintains the large difference in concentration of

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[66] E. B. Man, P. H. Lavietes, and D. S. Riggs, *J. Biol. Chem.*, **143**, 361 (1942).

[67] F. Joliot, *Proc. Roy. Soc. London*, **A184**, 1 (1945).

[68] H. Davson, Cold Spring Harbor *Symposia of Quant. Biol.*, **8**, 255 (1940).

[69] G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **16**, No. 1 (1941).

[70] L. Hahn, G. Hevesy, and O. Rebbe, *Biochem. J.*, **33**, 1549 (1939).

[71] P. K. Smith, A. J. Eisenman, and A. W. Winkler, *J. Biol. Chem.*, **141**, 555 (1941).

[72] L. J. Mullins, W. O. Fenn, T. R. Noonan, and L. Haege, *Am. J. Physiol.*, **135**, 93 (1941).

[73] H. Levi, *Kgl. Danske Videnskab. Selskab Mat. fys. Medd.*, **23**, No. 10 (1945).

[74] R. B. Dean, T. R. Noonan, L. Haege, and W. O. Fenn, *J. Gen. Physiol.*, **24**, 353 (1941).

[74a] W. E. Cohn and E. T. Cohn, *Proc. Soc. Exptl. Biol. Med.*, **41**, 445 (1939).

potassium and sodium inside and outside the tissue cells. If the affinity of potassium for some constituents of the corpuscle or the plasma were much different from the affinity of sodium for these constituents, the difference in the potassium and sodium concentration of the corpuscles and the plasma could easily be explained. However, the high potassium

TABLE 201

Sodium and Potassium Content of Red Corpuscles of Various Vertebrates[75]

Animal	Sodium	Potassium	Na + K, mM/100 g. corpuscles
Carnivores			
Dog.............	10.7	0.87	11.57
Cat.............	10.4	0.59	10.96
Rodents			
Rabbit...........	1.60	9.91	11.51
Mole rat..........		10.45	
Albino rat.........	1.19	10.05	11.24
Guinea pig.........	1.50	10.45	11.95
Primates			
Man[76]...........	1.48	9.70; 8.2[74]	11.18
Monkey..........		11.15	
Ungulates			
Pig..............	1.08	9.95	
Ox..............	7.90	2.18	
Goat.............	9.32	1.84	
Gazelle...........	7.52	3.75	
Sheep............	3.50	5.26	8.76
Camel............	1.96	5.0	6.97
Horse............		8.77	
Mule.............		9.21	
Birds			
Chicken..........	0.71	9.73	10.44
Turkey...........	0.97	9.95	10.92
Guinea hen........	1.03	10.56	11.59
Partridge.........		12.87	
Goose............	0.68	8.99	9.67

[75] S. E. Kerr, *J. Biol. Chem.*, **117**, 225 (1937).
[76] G. M. Streef, *J. Biol. Chem.*, **129**, 661 (1939).

concentration in the corpuscles and the magnitude of the osmotic pressure of the plasma are difficult to reconcile with the occurrence of the whole or a large fraction of the K as free K^+. These considerations led Krogh[77] to advance the view that the difference in the chemical affinity of potassium and sodium exerts itself in the corpuscle membrane. The ions to be transported are assumed to enter a fairly stable and specific combination with organic molecules in the very surface of the protoplasmic membrane of each cell. As Krogh emphasized, it is convenient, even if the true mechanism may be quite different, to visualize the boundary layer surrounding each cell as representing the medium through which the exchange takes place according to the model proposed by Lundegårdh which shows essential similarities to the Langmuir film. Lundegårdh considers the boundary layer of plant cells a mosaic containing both indifferent micelles and others spaced at certain intervals which have definite affinities at one or both ends.

For the plant cells studied by Lundegårdh it is only necessary to assume different micelles with cation- and anion-binding powers, but for corpuscles it is necessary to postulate much more specialized micelles capable of binding specifically K^+ or Na^+. Just as in Langmuir films, the single micelles occasionally turn, and an ion combined to one end, say at the outside, will at intervals be present on the inside face and may be split off. Such a structure may account for the exchange of ions between corpuscles and plasma without leading to loss of the potassium excess present in the corpuscles. An exchange without change in the concentration of the ions may also take place if the difference in concentration is maintained by an electric potential difference, for example, in the case of a Donnan equilibrium.[78]

The mechanism responsible for the maintenance of the high concentration of potassium inside the corpuscles remains effective only as long as metabolic processes, in which mainly a nonoxidative breakdown of carbohydrate is involved, are going on in the corpuscles. When human blood is stored at 2–5° C., for example, for some time a large part of the excess potassium of the corpuscles leaks out. When the temperature of the blood is raised, the original state of potassium distribution is more or less restored and the accumulating mechanism again becomes effective. Corpuscles which continue to carry on glycolysis maintain their potassium content longer than do those in which the

[77] A. Krogh, *Proc. Roy. Soc. London*, **B133**, 140 (1946).
[78] H. Ussing, *Nature*, **160**, 262 (1947).

glucose becomes depleted. In blood stored at 2–5° with glucose, the erythrocytes lose less potassium than when no glucose is added and, similarly, the amount of potassium re-entering the cells from 10-day-old stored blood without glucose is increased if glucose is added.[77,79] These facts illustrate the close connection between corpuscular metabolism and the concentration of potassium in the corpuscles.

However, that potassium can enter organic compounds, even if only transitorily, is shown by the fact that potassium ion has a specific effect on the phosphorylation of the adenylic system in muscle and has an accelerating effect on the transfer of phosphate from 2-phosphopyruvate to the adenylic system.[80] Such an effect is difficult to explain without assuming participation of potassium in these reactions (cf. page 384).

B. RATE OF PENETRATION OF LABELED POTASSIUM INTO RED CORPUSCLES

A detailed investigation of the rate of penetration of labeled potassium into corpuscles was carried out by Mullins and co-workers[72] (see
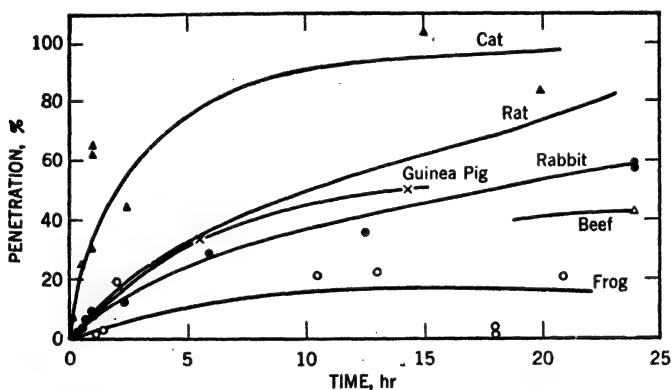
Fig. 95. Extent of interchange between potassium ions of plasma and red corpuscles.[72]

also Dean et al.[74]). A summary of their results is shown in Figure 95 and Table 202. In the case of the corpuscles of the cat, which have a low potassium content, a 100% partition of ^{42}K between corpuscle potassium and plasma potassium is obtained in the course of 15 hours

[79] J. E. Harris, J. Biol. Chem., 140, liii (1941). T. S. Danowski, ibid., 139, 693 (1941).

[80] P. D. Boyer, H. A. Lardy, and H. P. Phillips, J. Biol. Chem., 149, 529 (1943)

(see also Hahn and Hevesy[11]). In rabbit corpuscles about 50% partition is observed in the course of 1 day, the extent of partition increasing only slowly in the later phases of the experiment (see also Hevesy and Hahn[81]). In the frog (Fig. 95) no indication of a further interchange between corpuscle and plasma potassium is observed after the lapse of 10 hours, although the extent of interchange reached amounts to only 20%.

In addition to volume (V), surface (S), and potassium content, the time for 30% exchange and turnover are recorded in Table 202. Cat erythrocytes exchange 45% of their potassium per hour, frog cells only 1.4%, while others exchange 4 to 10%. The fairly rapid rate of penetration of ^{42}K into human red corpuscles, determined by Mullins and associates, is shown in Table 203.

TABLE 202

Permeability of Red Corpuscles to Potassium[72]

Animal	$V \times 10^{-12}, \mu^3$	$S \times 10^{-8}, \mu^2$	K content, m.e.q./l.	Time for 30% exchange, hr.	Turnover, 100 pS/KV
Dog.........	57	96	7.7	3.4	10.3
Cat.........	30	62	12.0	0.8	45
Rabbit	54	93	90	7.0	5.0
Man........	78	119	85	8.2	4.3
Guinea pig ...	58	96	89	4.5	7.8
Rat........	48	86	90	4.5	8.0
Frog........	4015	942	76		1.4

In interpreting these figures we must take into account that the penetration figures were obtained by comparing the activity of 1 mg. of corpuscle potassium and 1 mg. of plasma potassium at the end of the experiment, and, correspondingly, the penetration of most of the ^{42}K present in the corpuscles took place from the plasma, which contained appreciably more active potassium than was found to be present at the end of the experiment. This fact leads to an overestimate of the rate of penetration of potassium into the corpuscles.

In experiments in which the change in potassium content of the red corpuscles following the change in potassium concentration of the plasma was investigated, results indicating small changes only were

[81] G. Hevesy and L. Hahn, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **16**, No. 1 (1941).

obtained.   When the potassium concentration of human plasma was raised to 4–15 times its normal value without changing the total cation concentration, the amount of potassium penetrating the corpuscles in the course of four hours at 38° C. was found to be negligible; the corresponding figure for the penetration of ^{42}K was 4.[71]   While the penetra-

TABLE 203

Penetration of ^{42}K into Human Red Corpuscles *in Vivo*[72]

Subject	KCl ingested		Time, hr.	Potassium, m.e.q./l.		Counts/liter[b]		Counts/K		Penetration, %
	Mg.	Activity[a]		Corpuscles	Plasma	Corpuscles	Plasma	Corpuscles	Plasma	
L.J.M.	150		10.8	73.7	3.46	858	91.4	11.6	26.4	44
T.R.N.	288	2.8	10.8	84.5	3.19	909	86.6	10.8	27.1	40
W.O.F.	218	8.0	12.0	57.7	4.01	233	47.4	4.04	11.8	34

[a] Counts per minute $\times 10^{-6}$.
[b] Counts per liter are given in arbitrary numbers.

tion of ^{42}K into the corpuscles in these experiments is compensated by loss of an equivalent amount of "stable" potassium, a process which takes place promptly, the entrance of excess potassium must be compensated by a loss of an equivalent amount of sodium or other permeating cations present in small concentration in the human corpuscles.   This takes place to a very restricted extent, only.

While the red corpuscles concentrate potassium to a very remarkable extent, their potassium uptake is not increased appreciably by increasing the concentration of plasma potassium.   This fact explains why the pronounced permeability of red corpuscles to potassium is not revealed by the usual analytical methods, but is strikingly shown when investigating the interchange of corpuscle and plasma potassium using a radioactive indicator.

In the corpuscles of the dog, the potassium content is only one-tenth that of human corpuscles, but the sodium content is correspondingly higher (see Table 201).   Excess potassium added to the plasma is found to penetrate the corpuscles easily, an eightfold increase in potassium concentration in the plasma being followed within 2 hours by an almost 100% increase in the potassium content of the corpuscles.[82]

That an increase in potassium content of the plasma leads to a pronounced decrease in the percentage ^{42}K moving into the corpuscles is indicated by the results obtained by Levi.[73]   In *in vitro* experiments she found a substantial decrease in the percentage ^{42}K penetrating human

[82] S. E. Kerr, *J. Biol. Chem.*, **85**, 47 (1929/30).

corpuscles when the potassium content of the plasma was increased. The addition of calcium to the plasma had a similar effect. Furthermore, bee venom, which has only a slight effect on the rate of entrance of ^{32}P into red corpuscles, was found to increase the ^{42}K penetration rate appreciably.[83]

Since the percentage potassium penetrating the corpuscles from a concentrated potassium solution is smaller than from a dilute solution, the percentage ^{42}K moving from the plasma into the corpuscles will be smaller as well. Potassium thus shows an entirely different behavior from phosphate; a 25-fold increase in the phosphate concentration of the plasma is without effect (see page 459) on the percentage penetration of ^{32}P into the corpuscles.

The observation mentioned above — that an appreciable increase in the potassium content of the plasma leads to only minor changes in the potassium concentration of erythrocytes — and the fact that the concentration of potassium in the red corpuscles of most species is very much higher than its concentration in the plasma are responsible for the view generally accepted prior to application of labeled potassium in permeability studies. This is the view that red corpuscles are impermeable to potassium and sodium. The entrance of chloride, bicarbonate, phosphate, and other anions is a migration process of a more general type; not so the penetration of potassium. The chance that a potassium atom present in the red corpuscles will penetrate the corpuscle membrane is much smaller than the chance that a plasma potassium atom will penetrate in the opposite direction during the same time. The mechanism of this obstruction to the movement of corpuscle potassium atoms is not known. The obstruction is, as mentioned above, intimately connected with the carbohydrate metabolism taking place in the red corpuscles.

In early experiments,[35,70,81,84] only potassium of low and partly very low[70] specific activity was available, and, consequently, the potassium content of the plasma was raised appreciably by the addition of labeled potassium. The low penetration values obtained in these experiments may, at least partly, be explained by the high potassium content of the plasma. This fact illustrates the importance of the application of practically weightless isotopes as indicators. However, the penetration values obtained by Mullins and co-workers using potassium of very high specific activity are even higher than those obtained by Levi,

[83] H. Levi, *Svenska Vetenskapskad. Arkiv Kemi*, **A21**, No. 5 (1945).

[84] W. E. Cohn, *Am. J. Physiol.*, **133**, 242 (1941).

and may be taken to indicate correct values for the rate of potassium interchange. These penetration values do not differ much from the data obtained in experiments in which labeled corpuscles were shaken with nonlabeled plasma. Using labeled human corpuscles at 37° C., 5% of the [42]K present in the corpuscles was found to move into the plasma in two hours.[73] The rate of penetration of [42]K into the corpuscles is thus larger than the corresponding value for [32]P.

C. RATE OF PENETRATION OF LABELED SODIUM INTO RED CORPUSCLES

In early experiments,[70] labeled sodium was administered to rabbits and dogs[85] by subcutaneous injection of sodium chloride. After the lapse of one day, all or almost all the sodium of the corpuscles of the rabbit was found to be replaced; the [24]Na content of 1 g. corpuscle amounted to 14% of the [24]Na content of 1 g. plasma, a figure which is almost equal to the total sodium content of the corpuscles. In experiments both *in vitro* and *in vivo* on dog blood, Cohn[85] found that it requires twelve hours to obtain 50% sodium interchange between corpuscles and plasma. As found by Hahn and Hevesy[11] and by Mullins and colleagues,[72] 10–15% of the sodium present in the corpuscles of the cat is replaced in one hour.

From [24]Na added to human plasma 9% was found to penetrate into the corpuscles in the course of 4 hours.[71]

Krogh[77] compared the permeability of red corpuscles to radio-potassium and radiosodium by making use of the formula:

$$P = \frac{2.3 \, v}{s} \frac{\log q}{t}$$

where v is the volume, s is the surface of the corpuscle, and:

$$q = \frac{C_i}{C_i - C_o \, (a_i/a_o)}$$

C_i and C_o are the molal concentrations of the ion in question, inside and outside the cell, and a_i and a_o the corresponding radioactivities. The activity a_o should remain the same during the experimental period, while a_i corresponds to the end of the period. When v and s are measured in cubic centimeters and square centimeters, respectively, and t in hours, P is expressed in centimeters per hour. The sodium permeability is

[85] W. E. Cohn and E. T. Cohn, *Proc. Soc. Exptl. Biol. Med.*, **41**, 445 (1939).

calculated to be about 10^{-4} for the rabbit and 1.7×10^{-5} for the dog. Lower figures are obtained for the permeability of potassium. The above formula and those of other authors[72,86] permit calculation of the rate of potassium interchange, for example, between corpuscles and plasma. When we are faced with the problem of calculating the additional accumulation of potassium in the corpuscles following an increase in the potassium concentration of the plasma we cannot avail ourselves of the above-mentioned formulas which are based on the assumption that we are dealing with a problem of classical diffusion (see page 488 and Ussing[87]).

XI. Iron

A. GENERAL CONSIDERATIONS

The rate of penetration of iron ions into red corpuscles is unknown. The iron content of the corpuscle is to a very large extent composed of hemoglobin iron, which does not penetrate from the plasma into the corpuscles, but is incorporated in the corpuscles during their formation in the bone marrow. When corpuscles are shaken with plasma containing radioiron for 24 hours at 37° C., no radioiron was found in the corpuscles.[88,89]

When blood containing labeled red corpuscles is shaken with non-active dog plasma at 37° C., less than 15% of the labeled hemoglobin iron is found to be given off to the plasma within 24 hours.[88-90]

The iron atoms of the corpuscles have an almost unique position in view of the fact that they are not replaced during the lifetime of the corpuscles. The great stability of iron-labeled corpuscles is of great use in performing experiments of long duration with marked corpuscles. The stability of iron-labeled corpuscles, however, has the drawback that such red corpuscles cannot be obtained *in vitro*. As described on page 469, the phosphorus atoms of the red corpuscles of a patient can be labeled by shaking a blood sample of the patient with labeled phosphate and by reinjecting the marked sample into the patient we can

[86] R. B. Dean, T. R. Noonan, L. Haege, and W. O. Fenn, *J. Gen. Physiol.*, **24**, 353 (1941).

[87] H. Ussing, *Nature*, **160**, 262 (1947).

[88] P. F. Hahn, W. F. Bale, J. F. Ross, R. A. Hettig, and G. H. Whipple, *Science*, **92**, 131 (1940).

[89] J. Govaerts and A. Lambrechts, *Acta biologica belgica*, **3–4**, 209 (1943).

[90] P. F. Hahn, W. F. Bale, J. F. Ross, R. A. Hettig, and G. H. Whipple, *J. Exptl. Med.*, **76**, 21 (1942).

determine the circulating corpuscle volume of the patient or the rate of circulation of the corpuscles. In the performance of similar experiments with iron-marked corpuscles we have to dispose of a donor organism.

Iron-labeled red corpuscles are best obtained by administering ^{59}Fe after bleeding. Four hours after the administration of ^{59}Fe to dogs made anemic by bleeding some radioiron is already found in the red corpuscles,[91] and after the lapse of 4 to 7 days practically all ^{59}Fe absorbed is found in the erythrocytes. If the dietary conditions are favorable and small amounts of tagged iron are fed, practically all ^{59}Fe administered is already found in the red corpuscles of the dog in 2–3 days (see also page 168).

The incorporation of absorbed ^{59}Fe into red corpuscles is so pronounced that the ^{59}Fe content of the corpuscles is used as a measure of the amount of iron absorbed, by Balfour and colleagues[92] in their investigation of iron absorption by patients suffering from various diseases (see page 175).

Radioiron appears in the easily split fraction of the corpuscles in quantities ranging from 3–24% of corpuscle radioiron. No indication is found that the easily split iron fraction of red corpuscles is associated with any particular stage in the aging of the corpuscles.[93] Labeled erythrocytes in the circulation shortly after injection of radioiron (12 hours to 3.5 days) are markedly less resistant to hypotonic salt solution than are older erythrocytes. The difference in susceptibility disappears after a period of about 3 to 4 days.[93a]

B. DETERMINATION OF CIRCULATING CORPUSCLE VOLUME BY USE OF RED CORPUSCLES CONTAINING RADIOIRON

In the determination of circulating blood volume, a known volume of tagged corpuscles is introduced into the circulation. Blood samples are taken after homogenous mixing of injected and circulating blood is obtained. By comparing the activity of the injected and the secured

[91] P. F. Hahn, J. F. Ross, W. F. Bale, and G. H. Whipple, *J. Exptl. Med.*, **71**, 731 (1940).

[92] W. M. Balfour, P. F. Hahn, W. F. Bale, W. T. Pommerenke, and G. H. Whipple, *J. Exptl. Med.*, **76**, 15 (1942).

[93] L. L. Miller and P. F. Hahn, *J. Biol. Chem.*, **134**, 585 (1940).

[93a] W. O. Cruz, P. F. Hahn, W. F. Bale, and W. M. Balfour, *Am. J. Med. Sci.*, **202**, 157 (1941).

corpuscle samples, the circulating blood volume is calculated, as described on page 470. Results obtained by Hahn and co-workers[94] on the circulating red corpuscle volume of the dog are seen in Table 204. The

TABLE 204

Total Circulating Red Corpuscle Volume of the Dog Determined with Iron-Labeled Corpuscles[94]

Weight of dog, kg.	Total corpuscle vol. 10 min. after injecting labeled blood, ml.	Total corpuscle vol. 1–3 days after injecting labeled blood, ml.
12.6	290	280
13.8	445	405
8.0	255	250
17.0	465	515
10.7	380	340

average difference in the values obtained at 10 minutes and at three days after injecting the tagged corpuscles is only 3%. This result
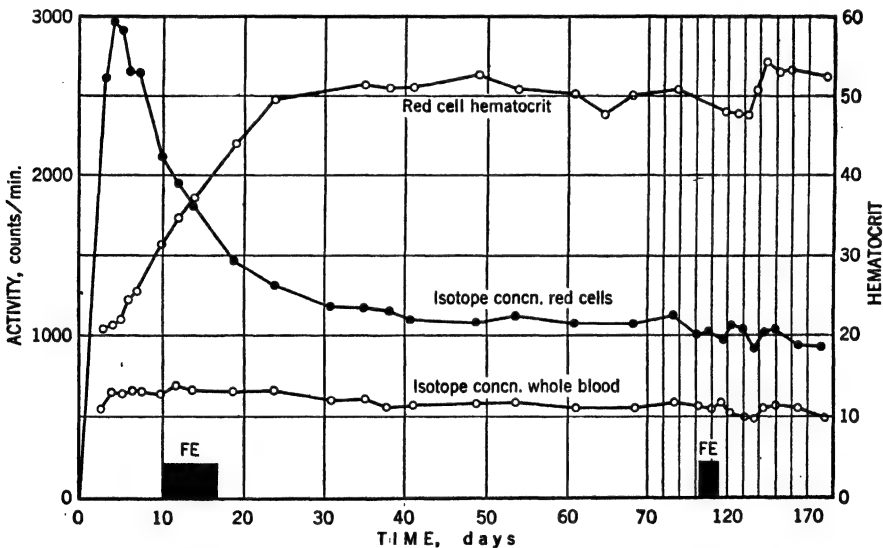
Fig. 96. Constancy of radioiron concentration of whole blood in an experiment lasting 170 days.[95]

indicates that in the usual healthy dog the number of immobilized red cells in marrow, spleen, and other vascular spaces does not exceed

[94] P. F. Hahn, W. M. Balfour, J. F. Ross, W. F. Bale, and G. H. Whipple, *Science*, **93**, 87 (1941).

[95] P. F. Hahn, W. F. Bale, and W. M. Balfour, *Am. J. Physiol.*, **135**, 600 (1942).

10% of the circulating mass and may be considerably less. The hematocrit value of the jugular blood in the above experiments averaged 46%. The hematocrit value for the whole body (determined by dividing the red corpuscle volume listed in the table by the sum of that volume and the determined plasma volume) is only 40%. This difference is considered due to the fact that the mean hematocrit of the capillaries, arterioles and venules is lower than the average body hematocrit. The average ratio of red corpuscle volume determined by the method of tagged corpuscles to red corpuscle volume calculated from plasma dye volume and jugular hematocrit is only 0.79.

When circulating erythrocytes were tagged by the incorporation of radioactive iron into their constituent hemoglobin, this iron may be followed in the blood, as shown by Hahn and colleagues,[95] for many months. This phenomenon is beautifully shown in Figure 96.

The total blood volume of the dog is maintained at a constant level, independent of the state of anemia. As the red corpuscle circulating volume increases, there is a corresponding drop in the plasma volume in order to maintain the total circulating blood volume at constant level.[95]

Hahn and Bale[96] found that a linear relationship exists between the red corpuscle volume and the jugular hematocrit over a range of hematocrit of 11–57%. For zero corpuscle mass, however, a hematocrit value of 8 is calculated.

In experiments on dogs, in contrast to humans, when the circulating red corpuscles are tagged with other dog corpuscles containing the radioactive isotope of iron, and nembutal anesthesia is induced, the removal of the engorged spleen shows that up to 30% of the circulating red corpuscles may be present in this organ.

Administration of adrenaline by vein results in an increase in the actively circulating red corpuscles; the increment in circulating corpuscles is found to be as much as 37% of the total red corpuscle mass. When adrenaline is administered to splenectomized animals the increased mass of circulating red corpuscles is about half as great as that obtained in the intact dog. This indicates that only about half the red corpuscles brought into the circulation under the influence of epinephrine originates from the spleen.[97]

[96] P. F. Hahn and W. F. Bale, *Am. J. Physiol.*, **136**, 314 (1942).

[97] P. F. Hahn, W. F. Bale, and J. F. Bonner, Jr., *Am. J. Physiol.*, **138**, 415 (1942/3).

Tagged red corpuscles were also used in the determination of the content of erythrocytes in a blood sample. To normal blood, labeled red corpuscles are added, and from the dilution of the activity the exact erythrocyte content is calculated. The red corpuscle content of the blood sample investigated is found to be 8.5% lower than the centrifuge hematocrit value. Centrifugation of blood as usually performed does not completely separate corpuscles from plasma and gives a value for the corpuscle volume which is too high.

Interesting information was obtained on the passage of the red corpuscles through the peritoneum and the lymph spaces by using tagged red corpuscles.[98] Red corpuscles injected intraperitoneally into the normal dog were found to pass without difficulty through the peritoneum and through the lymph spaces, lymphatic vessels, and lymph glands without injury or phagocytosis. In the normal dog 20 to 100% of the amount given within 24 hours is absorbed. Dogs made anemic by bleeding absorb tagged corpuscles less rapidly, the amount varying from 5 to 80% of the injected corpuscles.

C. DETERMINATION OF CIRCULATING RED CORPUSCLE VOLUME IN HUMANS

Beside the assumption that none of the radioactive atoms of iron pass out through the membrane of the intact erythrocytes, the method is based on the assumption as well that all the transfused tagged cells become thoroughly mixed with all of the recipient's cells, i.e., the ratio of radioactive to nonradioactive cells becomes and remains a constant throughout the entire vascular tree, and hence is independent of differences in hematocrit levels in large and small vessels.

The validity of the first assumption has been demonstrated by *in vitro* experiments. The validity of the second assumption has been established directly by *in vivo* experiments in humans. Compatible radioactive human erythrocytes were added to heparinized whole blood from a normal human. The experiment simulated the repeated determination of circulating red corpuscle volume before and after hemorrhage *in vivo*. The data from a typical experiment carried out by Gibson *et al.*[99] given in Table 205 show that recovery was complete within ± 3%.

[98] J. G. Gibson, A. M. Seligman, W. C. Peacock, J. C. Aub, J. Fine, and R. D. Evans, *J. Clin. Invest.*, **25**, 848 (1946).

[99] J. G. Gibson, S. Weiss, R. D. Evans, W. C. Peacock, J. W. Irvine, W. M. Good, and A. F. Kip, *J. Clin. Invest.*, **25**, 616 (1946).

In experiments with two moderately anemic patients the increase in red corpuscle volume as measured by the radioiron method before and after transfusions of fresh compatible blood was equal to the known amount of erythrocytes infused within $\pm 2\%$.[99]   The red corpuscle

TABLE 205

Recovery of Radioactive Red Corpuscles Added
to Nonradioactive Whole Human Blood[99]

| | Unit activities[a] | | |
Procedure	Calculated	Found	Recovery, %
Radioactive red corpuscles added........	0.647	0.672	103.5
Nonradioactive whole blood added.......	0.368	0.359	97.5
Whole blood removed and radioactive cells again added.........................	1.480	1.500	98.7

a Unit activity $= \dfrac{\text{counts/min./ml. corpuscles}}{\text{counts/min. of standard corpuscles}}$

volume transfused varied in these experiments between 400 and 800 ml. The red corpuscle volumes are calculated from the formula $V_{rr} = (C_D \times U_{aD}) / U_{aR}$ where V_{rr} is the red corpuscle volume, C_D is the number of milliliters of donor cells given, U_{aD}, the radioactivity of the donor corpuscles, and U_{aR} the radioactivity of the recipient cells. The labeled red corpuscles used in these experiments were obtained from donors of blood group O. About 1 mg. labeled iron as ferric ammonium citrate was given intravenously to the donor.

Radioactive red corpuscles are detectable in blood stream within 24 hours after administration of radioiron; the maximum level is attained in about 21 days. Thereafter the corpuscle radioactivity level fluctuates little, being affected chiefly by the decay of the isotopes, the withdrawal of red corpuscles, and the natural excretion of body iron. Bleeding prior to radioiron administration has been found to have little or no effect on utilization, even in individuals in normal iron balance, probably because the amounts given represent less than 1% of total iron stores. The maximum utilization was about 80%, the average utilization, 60%.

Each 10-ml. ampul used for the injections contained approximately 1 mg. of radioiron; this has been used as a standard dose. Specific activities of ^{55}Fe have ranged from 1×10^6 to 6×10^6 counts per minute per milligram iron per ampul, and of ^{59}Fe from 1×10^6 to 2×10^7. Thus preparation of donors can be accomplished in a short time with a few injections.

Red corpuscle samples containing a total of about 250 counts per minute above background were used. Since about 5 ml. of red corpuscles are taken for each sample, the radioactivity of the donor cells should be such that, after mixing, the recipient's blood contains about 50 counts per minute per milliliter of corpuscles.

For single volumes in normal adult humans, and infusion of from 70 to 100 ml. of whole donor blood (30 to 50 ml. of red corpuscles) having an activity of about 2500 counts per minute per milliliter will give satisfactory recipient cell radioactivity levels in individuals having red corpuscle volumes of from 1500 to 2500 ml.[99]

In experiments of Ross and Chapin,[100] in which the corpuscle volume of human subjects was determined by injecting corpuscles containing labeled iron, the corpuscle volume 10 minutes after the injection was found to be similar to that observed 24 hours after injection. It is believed that even if 10 minutes should be too short a period of time to allow mixing of tagged corpuscles with any considerable volume of immobilized cells, complete mixing would have occurred after 24 hours. The volume of red corpuscles in active circulation thus appears to be the same as the total volume of red corpuscle in the vascular system (including the splenic sinusoids), and no significant reserves of corpuscles can be demonstrated (see page 472). The total volume of corpuscles is not increased by administering adrenaline.

The red corpuscle content determined by making use of corpuscles labeled with radiophosphorus or radioiron is markedly lower than the corpuscle content obtained when estimated from venous hematocrit ratio and the plasma volume measured by blue dye dilution. The ratios obtained are seen in Table 206. A low ratio was also found by Hahn and co-workers (see page 494) when determining the corpuscle content of the dog.

TABLE 206

Ratio of Red Corpuscle Content of Patients as Determined
by Labeled Corpuscle and Dye-Hematocrit Methods

Corpuscles labeled with	Ratio
Radiophosphorus (Hevesy et al.[56]) (average of 19 patients)	0.85
Radioiron (Gibson et al.[50d]) (cf. also Gibson et al.[98])	0.85
Radioiron (Meneely et al.[50c]) (average of 24 patients)	0.80

[100] J. F. Ross and M. A. Chapin, *J. Clin. Invest.*, **21**, 640 (1942).

TABLE 207

Average Values for Intravascular Contents of Small and Large Vessels of Various Organs of Seven Dogs[98]

Organ	Per cent of body weight	Intravascular content of small vessels, ml. per g. tissue			Per cent of total circulating volume			Hematocrit of blood in small vessels		Ratio of rapidly circulating to total corpuscles
		Red corpuscles	Plasma	Whole blood	Red corpuscles	Plasma	Whole blood	Range, %	Average,[a] %	
Spleen	0.4	0.367	0.065	0.420	5.1	0.4	1.5	55–100	82	0.97
Liver	2.7	0.048	0.115	0.200	4.7	5.2	4.9	14–58	41	1.06
Lungs	0.7	0.063	0.115	0.195	1.1	1.6	1.4	9–44	33	0.95
Kidneys	0.7	0.037	0.174	0.205	0.5	1.7	1.2	9–22	15	0.99
Heart	0.8	0.022	0.049	0.066	0.3	0.6	0.5	17–28	22	1.03
Bowel	3.5	0.006	0.050	0.060	1.0	2.9	1.5	8–30	17	1.05
Muscle	33.3[b]	0.004	0.014	0.018	3.4	7.5	5.8	8–32	21	1.04
Brain	0.6	0.006	0.01	0.013	0.4	0.5	0.4	16–21	18	
Total	42.7				16.5	20.4	17.2			

[a] Weighted average. Average hematocrit of auricular blood: range from 33.7 to 50.0%; average hematocrit of all circulating blood in large vessels: range from 32.9 to 47.3%; average hematocrit of all circulating blood: range from 31.9 to 44.3%.
[b] Estimated from value in 2 dogs in which all muscle was removed from skeleton.

D. DISTRIBUTION OF RED CORPUSCLES IN LARGE
AND MINUTE VESSELS

The volume of circulating red corpuscles labeled by the presence of radiophosphorus or radioiron is less than when measured at the same time in the same subject by the dye-plasma hematocrit method as described above. The result suggests that the hematocrit of all circulating blood in the body is normally lower than that of blood drawn from large arteries or veins, or from the right auricle. This suggestion induced Gibson and associates[98] to determine the distribution of red corpuscles and plasma in large and minute vessels of the normal dog. Plasma iodinated with radioactive iodine (see Fine and Seligman[101]) was used to determine the amount of plasma present, radioiron-tagged red corpuscles were used in the determination of the corpuscle volume.

The plasma volume of fasting dogs was determined by the usual dye method. At the same time the dog received an infusion of freshly drawn compatible dog red corpuscles labeled with ^{55}Fe for the determination of the circulating red corpuscle volume. Three to five hours later plasma volume was again determined, and an infusion of freshly drawn compatible dog red corpuscles labeled with ^{59}Fe was given. Also at the same time an infusion of albumin of fresh dog plasma iodinated with radioiodine (^{131}I) was given intravenously. Samples of blood were taken throughout a 1-hour period for measurement of plasma dye level and iodine radioactivity, measurement of the hematocrit and hemoglobin concentration of whole blood in the large vessels, and of the radioactivity level of both isotopes of iron in the red corpuscles.

The animals were autopsied immediately after death, and the weight of the organs was recorded. Organs analyzed were spleen, liver, lung, kidneys, heart, bowel, muscle, and brain. No bone marrow was obtained. The organs were not perfused, and no attempt was made to prevent blood from oozing out as the organs were cut up. Small pieces of tissue which avoided large vessels were gently wiped with gauze and weighed.

Representative samples of each organ were taken and finely divided. An aliquot of each organ was analyzed for hemoglobin, radioactive iodine, and both isotopes of radioactive iron.

The unit quantity of total red corpuscles was based upon the relative concentration of hemoglobin in whole blood and that obtained by extraction from tissue samples. The unit quantity of corpuscles tagged with ^{55}Fe and ^{59}Fe, respectively, was based upon the relative radioiron

[101] J. Fine and A. M. Seligman, *J. Clin. Invest.*, **23**, 720 (1944).

activities of both isotopes of a red corpuscle sample drawn just prior to death, and the tissue sample. In making radioiodine measurements in tissue, corrections were made for radioactivity due to radioiron from red corpuscles, but these were not significantly great in most instances. In 4 experiments, total red corpuscle content was calculated from extracted hemoglobin values, and in 3 experiments from ^{55}Fe values. Rapidly circulating red corpuscle content was based upon ^{59}Fe measurements in all instances. Total plasma and red corpuscle content was taken as the product of unit content and organ weight. Iodine measurements reflect only circulating plasma.

The net amount of whole blood removed in sampling was less than 5% of the initial total blood volume and no significant changes in mean arterial pressure were observed. No correction of tissue values for bleeding were made.

Table 207 summarizes the findings obtained in seven normal dogs.

It is seen that the hematocrit of the blood in the large vessels, of all the blood in the body, and of the blood in minute vessels, is always less than that of arterial or venous blood. The ratios of partition hematocrits to arterial hematocrit are approximately 0.9, 0.85, and 0.7, respectively. As to the ratio of rapidly circulating to total red corpuscles, it is unity for the organ investigated. Hence all red corpuscles in the vascular bed are in active circulation.

Plasma volumes calculated in the investigation described from radioiodoprotein data were found to be within ± 10% of the values obtained by the dye method. Dow et al.[102] made a direct approach to find out if dye T-1824 is retained during the passage of the dyed plasma through the lungs or other organs. They injected into the superior vena cava of dogs red corpuscles labeled with radioiron suspended in plasma dyed with T-1824. The entire left ventricular output was collected in a series of measured and mechanically timed samples, which were analyzed for dye and radioactivity. There was found no evidence that in comparison with the red corpuscles there was any preferential retention of dye in or on the vessels of the lesser circulation. The simultaneous concentration curves for dye and tagged erythrocytes were practically identical, with the curve for erythrocytes always a few milliliters of flow ahead, indicating a more rapid transit of the red corpuscles in some part of the circuit.

[102] P. Dow, P. F. Hahn, and W. F. Hamilton, *Am. J. Physiol.*, **147**, 493 (1946).

E. SURVIVAL OF PRESERVED HUMAN ERYTHROCYTES
STORED AS WHOLE BLOOD

Erythrocytes tagged with the two isotopes of radioiron were applied by Gibson et al.[104-106] and with ^{59}Fe by Ross and Chapin[103] in the study of the post-transfusion survival of preserved erythrocytes stored as whole blood or in resuspension, after removal of plasma.

Nonviable stored human erythrocytes are rapidly removed from the blood stream after transfusion, the rate of removal of the nonviable red corpuscles varying inversely with the percentage survival of the transfused tagged erythrocytes.[104-106] At or above 80% survival, nonviable erythrocytes are completely removed in 24 hours. At any survival level the majority of nonviable erythrocytes are removed from the blood stream during the first 2 hours after transfusion.[105] It is therefore only the nonviable portion of the transfused corpuscles that need be considered in the study of the effects of the transfusion of stored blood.

Studies were made of several preservative solutions for whole blood, of electrolyte solutions for resuspension of centrifuged erythrocytes, electrolyte resuspension fluids to which human serum fractions were added, and of packed red corpuscles stored without resuspension after the removal of plasma. Some of the results obtained are seen in Table 208; the survival percentage varied between 100 and 17%.[104]

Refrigeration, the addition of dextrose to the citrate anticoagulant, the maintenance of a slightly acid reaction of the diluted plasma or resuspension fluid, and optimal dilution are found to be essentials for prolonged preservation. Whole blood can be preserved with up to 70% viability for at least 21 days in acid–citrate. Packed red corpuscles from blood drawn into acid–citrate–dextrose may be safely transfused without the addition of a dilutant after up to 21 days of storage.

Numerous data were collected on the effect of varying temperatures on the post-transfusion survival of whole blood during depot storage and after transportation by land and air.[106] Refrigeration is an absolute essential for preservation of human erythrocytes drawn as whole blood

[103] J. F. Ross and M. A. Chapin, J. Am. Med. Assoc., **123**, 827 (1943).

[104] J. G. Gibson, R. D. Evans, J. C. Aub, T. Sack, and W. C. Peacock, J. Clin. Invest., **26**, 715 (1947).

[105] J. G. Gibson, W. C. Peacock, R. D. Evans, T. Sack, and J. C. Aub, J. Clin. Invest., **26**, 739 (1947).

[106] J. G. Gibson, T. Sack, R. D. Evans, and W. C. Peacock, J. Clin. Invest., **26**, 747 (1947).

in acid–citrate–dextrose. The optimal range for depot storage lies between 4° and 10° C. Exposure to temperatures below − 4° C. or above 10° C. even for a 24-hour period, increase the rate of *in vitro* deterioration; and this rate is markedly accelerated at temperatures above 15°. Ross and Chapin[103] found that storage of citrated blood exerts a deleterious effect on the survival of transfused red corpuscles. The survival of these erythrocytes varies inversely with the duration of storage.

TABLE 208

Post-transfusion of Human Erythrocytes Drawn as Whole Blood
in 4% Sodium Citrate and Stored after Resuspension
in Electrolyte Solutions at 4° C.[104]

Resuspension solution	Days stored	Transfused volume		Age of tagged corpuscles		Survival, %
		Total, cc.	Corpuscles, cc.	^{55}Fe, days	^{59}Fe, days	
0.85% sodium chloride .	0	175	68	40		92
	3	160	68	40		90
	10	140	64	40		8
10% corn syrup........	0	59	31	24		100
	5	57	19	24		60
	12	62	20	24		42
	21	97	32	24		17
	9	502	227	9		95
	13	487	169	9		94
	15	475	178	11		41
	20	470	193	11		45
	11	479	189	8		92
	12	480	173	12		78
	14	520	220	10		60
	18	425	164	10		58
Phosphate-buffered citrate–dextrose, pH 6.8.............	2	74	30	88		98
	6	60	24	88		97
	11	67	26	88		88
	20	64	23	88		86
	3	134	58	133	28	90
	10	137	58	133	28	94
	16	131	56	133	28	80
	30	117	50	133	28	45

F. METABOLISM OF INORGANIC AND HEMOGLOBIN IRON

Uncertainty exists regarding the relative therapeutic effectiveness of iron administered by mouth and by parenteral injection. Furthermore, the immediate fate of the iron contained in transfused erythrocytes and in intravascularly liberated hemoglobin is obscure. Ross[106a] has investigated these problems with the radioactive iron technic. Inorganic salts of iron, normal human erythrocytes, and hemoglobin solutions labeled with radioactive iron have been administered to human subjects by mouth and by intravenous injection. He has found that the rate of utilization of iron is identical regardless of mode of administration. Thus the iron liberated from erythrocytes or hemoglobin destroyed after transfusion appears in the erythrocytes of the recipient just as rapidly but not more so than inorganic iron given by mouth or injection. Utilization is complete in approximately 21 days in all instances, and approximately 60% of the amount of iron absorbed appears in the circulating erythrocytes, an amount proportional to the percentage of total body iron existing in circulating erythrocytes.

These findings indicate that once iron has been introduced into the body it enters a "metabolic pool" of body iron and is metabolized in identical fashion regardless of its mode of entry.

G. RADIOIRON IN THE FETAL CIRCULATION

Radioiron fed in a single dose to women near termination of pregnancy appears rapidly in the fetal circulation, measurable amounts being present after 40 minutes. The speed of transfer suggests that plasma rather than the red corpuscle is the vehicle (*cf.* page 477). Distribution of ^{59}Fe in fetal tissues following feeding to the mother and subsequent therapeutic abortion show a wide distribution of the isotope with the greatest concentration in the red corpuscles. Among the other tissues studied the liver contains the largest quantity.[107]

H. PROBLEM OF THE LIFE CYCLE OF THE RED CORPUSCLES

· In view of the fact that ^{59}Fe atoms present in the corpuscle hemoglobin are not renewed during the lifetime of the corpuscle, it would be expected that iron-labeled red corpuscles can be used to determine the life cycle of the erythrocytes. This application of tagged corpuscles is

106a J. F. Ross, *J. Clin. Invest.*, **25**, 933 (1946).

107 W. T. Pommerenke, P. F. Hahn, W. F. Bale, and W. M. Balfour, *Am. J. Physiol.*, **137**, 164 (1942).

not feasible because of the tendency of the organism to avail itself of the iron-containing debris of decaying corpuscles in the formation of new erythrocytes, a fact closely connected with the unique tendency of the organism to conserve its iron contents.
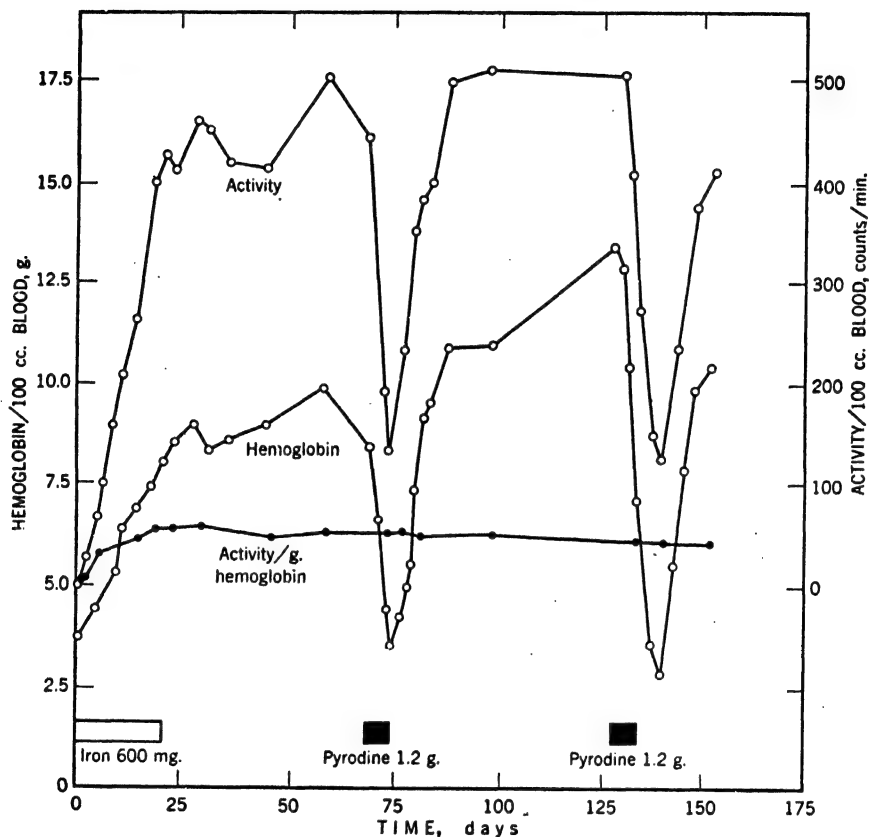
Fig. 97. Drop in hemoglobin and activity concentration of dog blood following administration of acetylphenylhydrazine (pyrodine).[108]

The iron liberated from the hemoglobin derived from red corpuscles destroyed by acetylphenylhydrazine is utilized readily and nearly quantitatively for the regeneration of hemoglobin in the new red corpuscles during the period of spontaneous recovery from anemia and, correspondingly, the activity per gram hemoglobin hardly changes within 150 days, can be seen in Figure 97. The resultant drop in hemoglobin concentration following administration of acetylphenylhydrazine and the parallel drop in activity concentration of the blood can also be seen in the figure.

Following the period of hemoglobin concentration during the regeneration phase it will be noted that the activity concentration again parallels the hemoglobin concentration.

When hemoglobin is destroyed, all of the pigment radical is excreted as bile pigment, but only 3% of the released iron is eliminated in the bile and the remainder is conserved. Biliary iron is not affected by intravenous colloidal iron or iron by mouth.[108]

When disintegration of the red corpuscles occurs either by aging or trauma, even in the presence of adequate inactive storage iron (430 mg. colloidal ferric hydroxide was, for example, given by vein to a dog) the labeled iron from the liberated hemoglobin is almost immediately reutilized by new corpuscles so that the total circulating radioactivity is maintained constant. Of 5 mg. intravenously administered radioactive iron, 15% appears in the circulation after the second day. From this data and the average lifetime of human erythrocytes (assumed to be 125 days), the labile pool of iron available is calculated to amount to 133 mg.[109]

From the above considerations it follows that it is not feasible to use erythrocytes labeled by the presence of radioiron in the determination of the life cycle of the red corpuscles.

I. AGE OF ERYTHROCYTES INVADED BY MALARIAL PARASITES

The age of red corpuscles preferentially invaded by the malarial parasite *Plasmodium vivax* was determined by using radioiron.[110] The principle of the method lies in the fact that radioactive iron administered intravenously is incorporated into the hemoglobin molecule and begins to appear within 24 hours in the hemoglobin of newly formed red corpuscles. Since radioactive iron is taken up by erythrocytes only during the period of their formation, the radioactivity of a blood sample is dependent upon the presence of erythrocytes formed subsequent to the time of radioactive iron administration.

Nineteen ml. of a solution of ferric ammonium citrate containing ^{55}Fe was injected intravenously on April 10, into a patient who had been inoculated with trophozoites of vivax malaria on April 4. Samples of blood for erythrocyte and parasite counts and for the preparation of parasite concentrates were taken on that day and on subsequent days.

[108] W. B. Hawkins and P. F. Hahn, *J. Exptl. Med.*, **80**, 31 (1944).

[109] G. R. Greenberg and M. M. Wintrobe, *J. Biol. Chem.*, **165**, 397 (1946).

[110] J. W. Ferrebee, J. G. Gibson, and W. C. Peacock, *J. Infectious Diseases*, **78**, 180 (1946).

Comparison was made of the radioactivity of samples of whole blood and the radioactivity of samples of parasite concentrates.

When compared on a unit basis the radioactivity of the concentrates exceeded the radioactivity of the whole blood 15 to 50 times. Since radioactivity in both cases was confined to young erythrocytes formed subsequent to the time of injection of the radioactive iron, the observation is interpreted to mean that the concentrates contained a selected group of young red corpuscles.

XII. Copper, Cobalt, Zinc, and Lead

When 0.8 mg. labeled copper is administered with each meal to a dog, the maximal tagged copper content is observed in the plasma after the lapse of 2–4 hours, while the radiocopper content of the red corpuscles continues to increase within the time of experiment (48 hours).[111] In anemic dogs the uptake of ^{64}Cu in the corpuscles is accelerated.

Negligible amounts of radiocobalt are incorporated during their formation or penetrate into the red corpuscles after subcutaneous injection of 250 micrograms of $^{56}Co + ^{58}Co$ to a dog weighing 10 kg. After the lapse of 1.5 hours, 1 ml. plasma contained 0.06% of the amount administered, the percentage present in the plasma declining to 1/6 and 1/35 after the lapse of 1 and 14 days, respectively.[112]

Two hours after subcutaneous administration of ^{65}Zn of negligible weight to a dog weighing 10 kg., the ^{65}Zn content of 1 g. red corpuscles was found to be 1/40 of the corresponding content of 1 g. plasma. After the lapse of a day the corresponding ratio was roughly 1 and, after the lapse of a fortnight, 7. During that interval the plasma declined to 1/400 of its 2-hour value which amounted to 0.02% of the ^{65}Zn injected per ml. plasma. The ^{65}Zn content of the corpuscles reached its peak value in the course of a fortnight.[112] (Cf. also Sheline et al.[112a])

By making use of radium D as a tracer it was found that about 90% of the lead administered by intravenous injection is present in the red corpuscles of the dog when centrifuging was begun at the end of 1 minute, and 97 to 99% after 10 or 15 minutes. The process is readily reversed in vivo.[113]

[111] H. Yoshikawa, P. F. Hahn, and W. F. Bale, *J. Exptl. Med.*, **75**, 489 (1942)

[112] G. Hevesy, *unpublished data.*

[112a] G. E. Sheline, I. L. Chaikoff, H. B. Jones, and M. L. Montgomery, *J. Biol. Chem.*, **149**, 139 (1943).

[113] R. A. Mortensen and K. E. Kellog, *J. Cellular Comp. Physiol.*, **23**, 11 (1944). See also B. Behrens and R. Pachur, *Arch. exptl. Path. Pharmakol.*, **122**, 317 (1927).

Shortcomings of Radioactive Indicators

I. General Remarks

An ideal isotopic indicator has to show, within the error of the experiment, a behavior identical with that of the isotope whose path it is to trace. The chief shortcomings of radioactive tracers are to be looked for in the chemical nonidentity of isotopes and in the biological effects of the radiation emitted by the tracer.

II. Chemical Nonidentity of Isotopes

Isotopes are not chemically identical bodies. This fact is most conspicuously shown by the isotopes of hydrogen. If the latter were chemically identical, the distribution coefficient of deuterium between hydrogen compounds and water should be one. However, a distribution coefficient of deuterium as low as 0.43 is found between ethyl mercaptan[1] and water, and a distribution coefficient as high as 1.11 between aniline[2] and water. Hydrogen has a unique position: it is the sole element encountered as a naked nucleus (although only transitorily) in a chemical reaction. Chemical forces do not suffice to remove all electrons from any other element. Differences in the structure of the nucleus will, therefore, make themselves more noticeable in the chemical behavior of hydrogen isotopes than in the case of any other element. Furthermore, the difference between the mass of the hydrogen atom and that of the deuterium atom is 100%, while the corresponding differences between the isotopes of other elements are much smaller. The $C - {}^1H$ bond is weaker than the $C - {}^2H$ or $C - {}^3H$ bond due to the higher zero point energy for the lighter isotope. The activation energy is lower for the same reason. Hydrogen-transferring enzymes are sensitive to small differences in activation energy, so that isotope differentiation can occur in enzyme reactions invalidating the application of deuteron or tritium as hydrogen tracers.[2a] The imperfection of deuterium as a tracer, how-

[1] C. K. Ingold and C. L. Wilson, *Z. Elektrochem.*, **44**, 62 (1938).

[2] M. Harada and T. Titani, *Bull. Chem. Soc. Japan*, **10**, 554 (1935); **11**, 465 (1936).

[2a] T. H. Norris, S. Ruben, and M. B. Allen, *J. Am. Chem. Soc.*, **64**, 3037 (1947).

ever, has not prevented its very extensive application, which has led to a series of most important findings.[3]

Less marked differences are found in the chemical properties of the isotopes of other elements.

The equilibrium constant of the reaction:

$$^{15}NH_3 \text{ (g.)} + {}^{14}NH_4^+ \text{ (aq.)} \longrightarrow {}^{14}NH_3 \text{ (g.)} + {}^{15}NH_4^+ \text{ (aq.)}$$
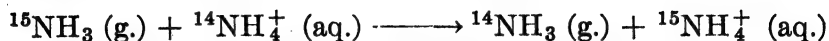
which was used by Urey[4] when concentrating the heavy isotope of nitrogen is only 3.3% smaller than unity, namely, 0.967. In the equation (g.) denotes gaseous and (aq.), aqueous.

The difference in the ratio of the distribution coefficients of ^{31}P and of ^{32}P between sodium phosphate and organic phosphorus compounds built up in an organism given labeled sodium phosphate is not known, but can be expected to lie within the errors of the experiment. Partly due to biological variations and partly to the errors involved in chemical operations and in the radioactive measurements, the accuracy of many data arrived at by making use of radioactive tracers amounts to as much as a few per cent or more. The accuracy of the data obtained may be expected to increase in the future, and the time may come when the difference from unity in the ratio of the distribution coefficients of ^{31}P and ^{32}P between different phosphorus compounds will have to be taken into consideration.

In the case of the potassium isotopes, such deviations were reported by some investigators to take place. Potassium as found in nature is a mixture of the stable isotopes ^{39}K and ^{41}K (ratio, 14:1) and the radioactive isotope ^{40}K (abundance 0.012%). Lasnitzki and Brewer,[5] making use of the mass spectrograph, investigated the distribution of the stable isotopes ^{39}K and ^{41}K in the potassium extracted from various tissues and from minerals. The same abundance ratio was found in all cases, except in the potassium extracted from the bone marrow and blood plasma. These contained 6.71 and 6.74% ^{41}K, respectively, while 6.52% was found in the potassium extracted from all other organs investigated. Lasnitzki and Brewer[6] found that in Jensen rat sarcoma the ratio of ^{39}K to ^{41}K was 7% larger than in normal tissue.

[3] R. Schoenheimer, *The Dynamic State of Body Constituents*. Harvard University Press, Cambridge, 1942.

[4] H. C. Urey, *Reports on Progress in Physics*, Vol. 6. Physical Society, London, 1939, p. 48.

[5] A. Lasnitzki and A. K. Brewer, *Biochem. J.*, **35**, 144 (1941).

[6] A. Lasnitzki and A. K. Brewer, *Cancer Research*, **1**, 776 (1941).

The ^{40}K content of potassium collected from human ashes was compared with the ^{40}K content of potassium prepared from inorganic sources, by measuring the radioactivity of the samples. The number of counts recovered per mole potassium by Fenn et al.[7] was on the average 2% lower in the human potassium. Changes in the proportion of ^{40}K in tumor tissue were not detected by Dounce.[8] Recently a comparison of the activity of potassium extracted from mineral and biological sources by Mullins and Zerahn has shown that their ^{40}K content is constant within 0.5%.[8a]

Even nonisotopic indicators, although showing some of the above-mentioned shortcomings of isotopic indicators to a much greater degree, may occasionally be used instead of isotopic indicators, when these are not available. In some cases, for example, bromine was used as an indicator for Cl, since radiochlorine, with its half-life of 38 min., found only restricted application as a tracer and ^{36}Cl which has a half-life of 10^6 years became only recently available. If bromine is administered to human and animal subjects, it is found that the same bromine-to-chlorine ratio prevails in the plasma and in all tissues, except the nervous tissue, the spinal fluid, and the thyroid. As an example may be mentioned the determination of the total chlorine content of a rabbit by administering a known amount of bromine and determining the bromine-to-chlorine ratio of the plasma.[9] From these data, the chlorine content of the rabbit body can be computed (the nervous tissue, thyroid, and spinal fluid forming only a small portion of the organism). The result obtained is 1.07 grams per kilogram body weight.

While nonisotopic indicators may be useful, they have to be used with much caution. Elaidic acid, for example, was employed with success by Sinclair[10] in the study of fat metabolism. But when rats were provided with large amounts of elaidic acid throughout the entire period of prenatal and postnatal development, the elaidic acid content of the fatty acids in the brain phosphatides was found to be only one-fourth that of the liver and the muscles. There is clearly a much greater degree of selection in building up the phosphatides of brain than of liver and muscle. This restricts the applicability of elaidic acid as an indicator (see page 328).

[7] W. O. Fenn, W. F. Bale, and L. J. Mullins. *J. Gen. Physiol.*, **25**, 345 (1942).

[8] A. L. Dounce, *J. Biol. Chem.*, **147**, 685 (1943).

[8a] L. J. Mullins and K. Zerahn, *personal communication.*

[9] E. G. Weir, *Am. J. Physiol.*, **127**, 338 (1939).

[10] R. G. Sinclair and C. Smith, *J. Biol. Chem.*, **121**, 361 (1937).

III. Noxious Effects of the Radiation Emitted
by Radioactive Indicators

Most of the artificially radioactive isotopes emit β-rays and some of them γ-rays; several of the natural isotopes emit α-rays. The α-ray emitting natural radioactive bodies emits partly β- or γ-rays (or both), but the emphasis lies on the emission of the strongly ionizing α-rays. All these ionizing radiations, if of sufficient intensity, may produce changes in the irradiated organism. Should that be the case, the radioactive tracer administered will indicate events not in the normal organism, but rather those which take place in the organism affected by irradiation.

When studying, within very wide limits, the percentage recovery of total ^{32}P and phosphatide ^{32}P in tissues of the mouse, Jones[11] found that the results were not influenced by variations in the radioactivity of ^{32}P. Five groups of tumor-bearing mice were injected with a solution of Na_2HPO_4 containing the same amount of ^{31}P, but different amounts of ^{32}P. The radioactivity varied from 1 to 70 microcuries, the last-mentioned dose being unusually high in tracer experiments on mice. In spite of the 70-fold difference in radioactivity of the dose employed, no difference was observed in the percentage of the administered dose recovered or as total ^{32}P in liver, tumor, blood, brain, and heart or as phosphatide ^{32}P in liver and tumor.

It is of interest to ask how many international Röntgen equivalents (r.) correspond to radiation emitted by 70 microcuries. Assuming that 1 microcurie of ^{32}P emits 3.7×10^4 β-particles per second, these ionizing particles produce, as shown by Marinelli[12] (see also Kenney[13]), in 1 gram of tissue in the course of a day, an ionization corresponding to 42.9 r. This result is obtained on the assumption that the ^{32}P is uniformly distributed in 1 g. of tissue and that we consider regions of tissue removed from the edges.

In experiments in which 70 microcuries is injected as sodium phosphate into a mouse, the liver takes up about 3.5 microcuries, an amount producing an ionization of 25 r. in 4 hours. Such a dose has no pronounced effect on oxidation and glycolytic processes in the organism and the results obtained by Jones et al. correspond to our expectation.

If 1 millicurie of ^{32}P is retained for twenty-four hours by an adult

[11] H. J. Jones, *personal communication.*
[12] L. D. Marinelli, *Am. J. Roentgenol. Radium Therapy,* **47,** 210 (1942).
[13] J. M. Kenney, *Cancer Research,* **2,** 130 (1942).

weighing 74 kg., the effect will be equivalent to 0.6 Röntgen equivalent of whole body radiation.

When 1 millicurie ^{32}P decays in a body weighing 70 kg. and, if we assume an equal distribution of the ^{32}P, each gram of the body gets a dose of about 8 r. These relationships, however, are certainly not exact since x-rays are usually administered from an external source to the surface of the body and penetrate to a variable depth, while the beta rays of ^{32}P are emitted from phosphorus actually in the tissues.[14] Of 1 millicurie of ^{32}P administered, part is excreted, and about two-thirds decays in the organism, reducing the above figure to about 5 r. Some parts of the organism take up much more ^{32}P than corresponds to an equal distribution; the ribs take up, for example, 4 times that amount[15] (*cf.* page 116). When administering 10 millicuries ^{32}P to a patient the accumulation of ^{32}P in the skeleton takes place successively only, the actual dose will be, even in the skeleton, below 200 r. Administration of ^{32}P having an activity of 540 microcuries was found to decrease the white corpuscle level of the patients.[16] From the facts accumulated regarding the lethal effects of fission products it can be estimated that man would be killed by about 10 microcuries per gram body weight.[17]

In experiments with a monkey, the administration of 2 millicuries per kilogram body weight was found to be lethal. The monkey died 47 days after administration of that dose. Marked hemorrhagic changes in the marrow as well as hemorrhagic areas under the skin and in the intestinal tract were noted.[18]

One microcurie of ^{32}P uniformly distributed through 1 ml. of culture solution was found to produce the some ionization in a 24-hour period as an exposure to 35 r. on living human lymphocytes and progranulocytes in marrow culture.[19]

A dose of 60 r. was found to influence slightly the mitotic rhythm of cell cultures of fibrocytes of the rabbit. Marshak and Walker[20] administered 7.6 microcuries after partial hepactectomy to 50-g. rats

[14] B. V. A. Low-Beer, J. H. Lawrence, and R. S. Stone, *Radiology,* **39,** 573 (1942).

[15] L. A. Erf, *Proc. Soc. Exptl. Biol. Med.,* **47,** 287 (1941).

[16] L. A. Erf and J. H. Lawrence, *J. Clin. Invest.* **20,** 567 (1941).

[17] R. S. Stone, *Proc. Am. Phil. Soc.,* **90,** 11 (1946).

[18] K. G. Scott and J. H. Lawrence, *Proc. Soc. Exptl. Biol. Med.,* **48,** 155 (1941). See also J. G. Hamilton, *Radiology,* **39,** 541 (1942).

[19] B. V. A. Low-Beer, H. G. Bell, H. J. McCorkle, and R. S. Stone, *Radiology,* **47,** 492 (1946).

[20] A. Marshak and A. C. Walker, *Am. J. Physiol.,* **143,** 226, 235 (1945).

and found the dose did not influence mitotic figures. Twenty-four hours later, 0.82% mitosis was found, while the controls showed 0.85%. Since the radiation emitted by the dose administered amounted to a few Röntgens only, this negative result corresponds to our expectation.

Anaphase figures of lymphosarcoma of the mouse are found to be reduced to 75% of their normal value by the action of 51 r., 3 hours after irradiation.[21] A dose of 51 r. is produced in the sarcoma of the mouse in experiments lasting 4 hours if about 140 microcuries are injected. Since part of the ^{32}P administered is excreted in 4 hours, we would have to administer somewhat more than 140 microcuries ^{32}P to obtain a reduction of the anaphase figures of the lymphosarcoma to 75% of their normal value. A dose of 140 microcuries ^{32}P is an extravagant one to use in tracer experiments on mice.

The rate of incorporation of ^{32}P into desoxyribonucleic acid of cell nuclei has been found to be reduced by 100 r. or more, as discussed on page 330. In view of the fact that in experiments lasting only a few hours the ^{32}P content of the nucleic acid P comprises only a very small percentage of the ^{32}P content of the total liver tissue (see page 326), the possible effect of 70 microcuries on the formation of labeled nucleic acid cannot be expected to influence the percentage recovery of total ^{32}P in the liver of the fully grown mouse; the same consideration applies to most of the other organs. After injecting colloidal chromic phosphate contai: ing ^{32}P into the circulation (see page 124), Jones et al.[11] observed that up to 95% of the colloid is recovered in the liver, where it remains, to a large extent, all through the life of the animal. In these experiments the animals survived when 150 microcuries or less were administered. The administration of .1 millicurie proved to be lethal within 10 days. The intensity of β-radiation in the liver and spleen was about 100 times the concentration in other tissues. A graphic model of the liver, elliptically shaped (2.9, 2.6 cm. diameters) and 0.60 cm. thick in the middle, tapered to the edge, gave an average distance of 0.2 cm. traversed by β-particles of internally produced β-radiation. Near the center of the liver, the energy absorbed is about 90% or 38 r. per microcurie per gram tissues per day. The liver on the average obtains 30.7 r. per microcurie per gram tissue per day. In the mouse about 27% of the β-radiation originating in the liver is absorbed by adjacent tissues up to 0.60 cm. away. In large animals, where the shortest thickness of these organs is 1 cm., relatively little external loss occurs. The forma-

[21] A. Marshak and M. Bradley, *Proc. Natl. Acad. Sci. U. S.*, **31**, 84 (1945).

tion of labeled desoxyribonucleic acid in the mammary tumor of mice was found to be appreciably reduced after administration of labeled chromic phosphate. Due to the accumulation of chromic phosphate in the liver, this organ received a dose of 3400 r. or more in the course of the experiment taking 4 to 48 hours. In experiments in which phosphatides were extracted 15 days after administration of inert radioactive colloids, the labeled sodium phosphate being injected 1 day prior to sacrificing the animal, no difference in the ^{32}P content of the total phosphatides extracted from the liver of these animals and of controls was found. In other experiments (see page 320) irradiation with 1000 r. was found to reduce the percentage renewal of liver phosphatides in experiments taking 2 hours.

When investigating the effect of immersing fragments of mouse sarcoma 180 in radioactive phosphorus solution prior to transplantation, the following results were obtained. Marked inhibition and restriction of growth were caused by exposure to ^{32}P solution of 100 and 125 microcuries per milliliters (about 50 and 75% inhibition, respectively). The viability of mouse sarcoma 180 was completely destroyed by immersion in ^{32}P solution of 150 microcuries per milliliter.[22]

Evans and Quimby,[23] injecting radioactive sodium into mice, had to administer 2.5 millicuries (83.5 microcuries per gram)[23] to obtain a shortening of the average survival days from 25 to 20 days. Results in reduction of white and red blood corpuscle counts and in shortening of life were found to be similar after whole body irradiation with Röntgen rays to the results obtained after administration of ^{24}Na. It has been found that 10 microcuries of radioactive sodium per gram body weight injected subcutaneously in the normal mouse is equivalent in effect to 100 r. of heavily filtered 200-kv. Röntgen rays. The equivalence of 10 microcuries administered per gram body weight to 100 r. is applicable only to mice, not to larger animals or to humans. In man, this amount would give a much higher dose of radiation, for two reasons. In the first place, whereas in the mouse practically no γ-radiation, and only about three-fourths of β-radiation, is effective, in the human practically all of the β-rays and a considerable portion of the γ-rays are absorbed. Furthermore, the mouse eliminates about one-third of the material in 3 days, while in this period the average human excretes less than 10%.

[22] K. Sugiura, *Cancer Research*, **2**, 19 (1942).

[23] T. C. Evans and E. H. Quimby, *Am. J. Roentgenol. Radium Therapy*, **55**, 55 (1946).

It is estimated that the "equivalent röntgens" from a given dose per gram body weight in the human would be at least twice as much as in a mouse. Minimum detectable effects on the blood picture of rats, mice, and rabbits were observed by Pecher after administration of 0.5 microcuries of radiostrontium per gram rat.[24] He estimates that an amount of radiation equivalent to 200–600 r. is given to the bony tissues when 1 millicurie strontium is intravenously injected in an adult. Brues et al.[25] found the acute lethal dose in mice to be 8 microcuries ^{89}Sr per mouse. Mice fed a normal diet containing 2.5 millicuries of ^{32}P died within 10 days.

The threshold dose of ^{32}P, so far as effect on formation of blood corpuscles is concerned, is said to be 0.006 to 0.009 microcurie per gram body weight.[26] 0.06 microcurie per gram body weight suffices to suppress the formation of additional erythrocytes, and may be used to cure polycythemia, as shown by Lawrence and associates.[27] Numerous cases of primary polycythemia are reported to be cured by intravenous injection of 5 millicuries ^{32}P; a summary of this work is given by Reinhard et al.[28] (see also Erf[29]). The threshold dose that produces erythema on the skin was found to be 34 microcurie hours per square centimeter.[30] Henshaw et al.[31] found in experiments on mice that survival time, which is one of the most sensitive responses, showed effects following daily exposures in the range of 0.1 n. of fast neutrons and 1 r. of gamma rays.

Both for the sake of economy and to avoid biological effects due to radiation emitted by the radioactive tracer, it is advisable to apply indicators of much restricted activity. This is especially the case in experiments with human subjects, since even when the result of the experiment is not influenced by the effect of radiation emitted by the tracer the organism may be injured.

A dose usually considered tolerable by human subjects is a dose of

[24] C. Pecher, *Univ. Calif. Berkeley Pubs. Pharmacol.*, **2**, 117 (1942).

[25] A. M. Brues, H. Lisco and M. Finkel, *personal communication*.

[26] B. V. A. Low-Beer and A. De G. Treadwell, *J. Lab. Clin. Med.* **27**, 1294 (1942).

[27] J. H. Lawrence, *in press*.

[28] E. H. Reinhard, C. V. Moore, O. S. Bierbaum, and S. Moore, *J. Lab. Clin. Med.*, **31**, 107 (1946).

[29] L. A. Erf, *Am. J. Med.* **1**, 362 (1946).

[30] E. E. Osgood, P. C. Aebersold, L. A. Erf, and E. A. Packham, *Am. J. Med. Sci.*, **204**, 372 (1942).

[31] P. S. Henshaw, E. F. Riley, and G. E. Stapleton, *personal communication*.

0.1 r. per day. The question of whether the gene injury produced by such a dose is significant or not cannot be considered, since sufficient data on human beings are not available to allow any satisfactory statement of how much gene damage is produced by this exposure, as emphasized by Henshaw.[32] This uncertainty is a further reason for using as low activities as possible in experiments with human beings and, as far as choice is possible, for choosing radioactive bodies of short half-life.

In view of the radiation effects, the experimenter must be aware of the hazards involved in handling radioactive isotopes. Application of restricted activities and extreme cleanliness minimize hazards, although volatile radioactive products, e.g., $^{14}CO_2$, may prove to be noxious even when inhaled in small amounts. Protection against radiation hazards is discussed in detail in the National Bureau of Standards Handbooks H23 and H20, *Radium Protection* and *X-Ray Protection*, respectively.

[32] P. S. Henshaw, *Radiology*, **46**, 62 (1946).

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